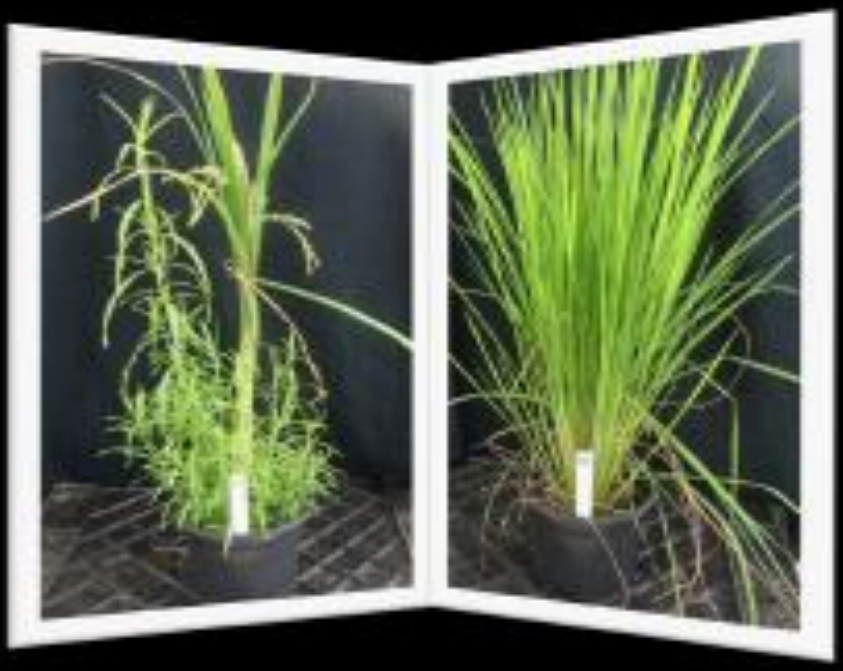
The relationship between strigolactones and *Striga hermonthica* infection in cereals



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The relationship between strigolactones and *Striga hermonthica* infection in cereals

Muhammad Jamil

Thesis

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“Vision without action is merely a dream. Action without vision just passes the time. Vision with action can change the world!”

Joel Arthur Barker

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To my beloved son M. A. Jamil

Chapter 1

General introduction

This thesis describes experimental work on rhizosphere signalling molecules, the "strigolactones", which are germination stimulants for the noxious parasitic weed, *Striga hermonthica* (*Striga*) in cereals. This introduction starts with a short overview of cereals and their production constraints. Then details on origin, distribution, damage mechanisms and life cycle of *Striga* are provided. The discovery of strigolactones, their biosynthesis and ecological significance as germination stimulants, hyphal branching factors for arbuscular mycorrhizal fungi as well as tillering/branching and root regulators is described. Finally, control strategies for *Striga* are introduced. Here the link between strigolactones and *Striga* infection, and how insights in this relation could lead to control strategies, is emphasized. The introduction concludes with a short overview of each of the chapters of this thesis.

Cereals: Global and African overview and production constraints

Cereals are members of the monocotyledonous grass or *Poaceae* family. They are mainly used as food, livestock and poultry feed and for industrial processing. The cereal grains are an important component of the daily diet of the majority of humans due to their high nutritional value consisting of about 70% carbohydrates, 10% protein and 3% lipids (Pomeranzy 1987). Wheat, maize and rice are the most widely grown cereals, accounting for 49% of the world's harvested area and 78% of the world's global cereal production and provide about half of the global requirements of calories and protein. During 2009 the global area planted with these (FAO 2009) major cereals crops was about 616 million hectares with a total production of 2.3 billion tons (Fig. 1). During the past 20 years about 4% increase in area and 40% increase in production and 27% increase in yield were achieved (Fig. 2). The world population increased from 3 billion to 6.6 billion between 1960 and 2006 (UNFPA 2008), and food consumption measured in kcals per capita increased from 2358 to 2803 between 1964 and 1999 (WHO 2008).

The most widely cultivated cereal crops in Africa include maize, sorghum, millet, wheat and rice (30, 24, 21, 10, 9 million ha, respectively) and their per capita consumption is increasing every year (5% per annum) (Sauerborn et al. 2000). In 2009 the total area for these crops in Africa was 14% of the global cereal production area while the total production of this region was only 5% of the world's cereal production. Over the past 20 years cereal production in Africa has increased steadily from 79 million tonnes in 1989 to 119 million tonnes in 2009 (FAO 2009) (Fig. 1). However, this increase in production can be attributed to an increase in the total area of cereals harvested (30%) in this period more than an improvement in productivity (Fig. 2).

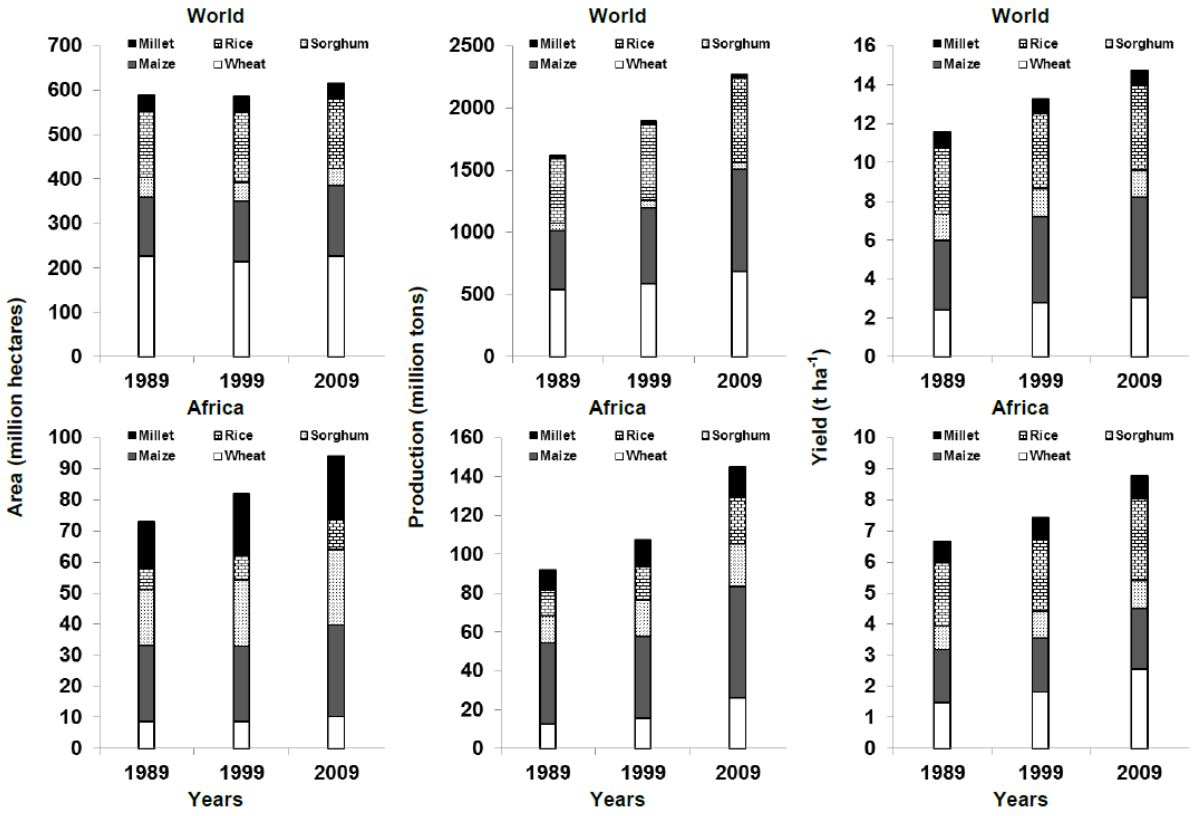

Fig. 1 Area, production and yield of the five major cereal crops in the world and in Africa during the past 20 years (1989-2009) (FAO 2009)

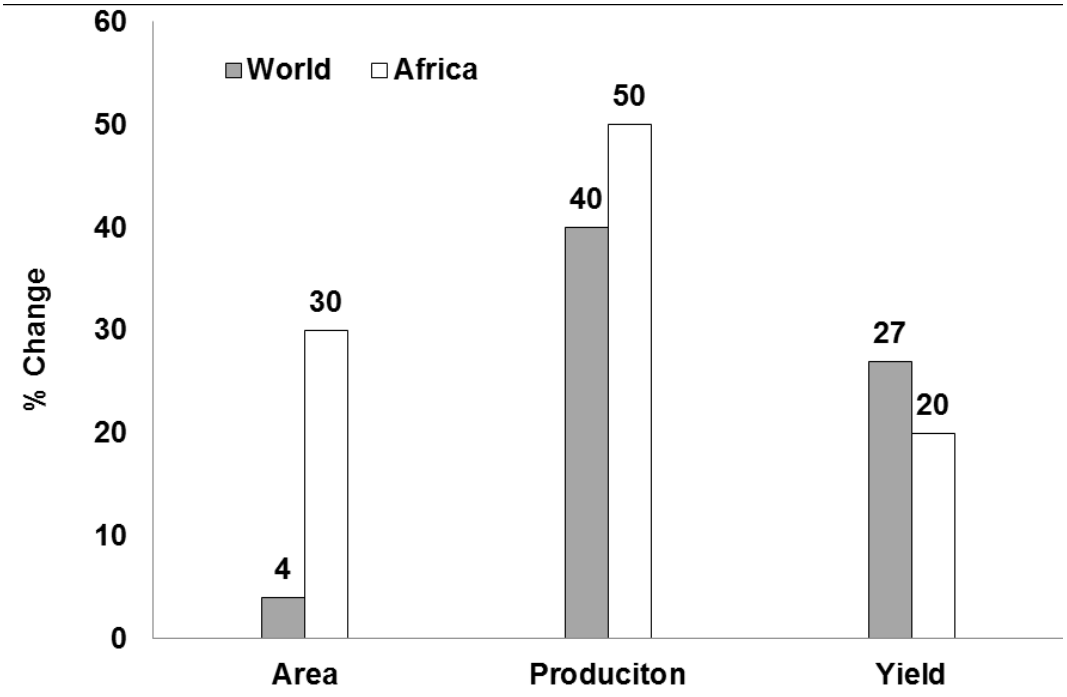

Fig. 2 Change (%) in area, production and yield of cereal crops in World and Africa during last 20 years (1989-2009) (FAO 2009)

The average yield of maize (1.9 t ha^{-1}), sorghum (0.9 t ha^{-1}) and rice (2.6 t ha^{-1}) in Africa is much lower than the world averages (5.2, 1.4 and 4.3 t ha^{-1} respectively) (Fig. 1). The main constraints limiting cereal production in Africa are inefficient management, poor economic conditions of the farmers, soil degradation (e.g. nutrient depletion, loss of organic matter), unavailability of fertilizers, drought and erratic rainfall, pests and diseases. Weed infestation is another one of the main causes of low cereal yields in Africa. Weeds can reduce yields by 50% up to complete crop failure (Rodenburg and Johnson 2009; Parker 2009). An important group of weeds on cereals in Africa, causing huge economic losses, are the parasitic weeds (Adetimirin et al. 2000; Gethi and Smith 2004; Kanampiu et al. 2003; Khan et al. 2006b). *Striga* spp., one of the major genera of parasitic weeds, infest about 40% of the cereal-producing areas of Africa resulting in crop losses estimated at US$7 billion annually, affecting the livelihood of approximately 300 million people (Ejeta 2007; Gressel et al. 2004). The subsistence farmers mostly affected by this weed may lose 20–80% of their yield (Gethi et al. 2005; Verkleij and Kuiper 2000), depending on the level of resistance and tolerance of the specific host genotype (Rodenburg et al. 2005). The greatest damage occurs in the Sahelian and Savannah zones of Africa, which constitute the major areas of cereal food production (Lagoke et al. 1991; Weber et al. 1995). The *Striga* problem is expected to grow which is strongly supported by the adaptive ability of *Striga* to new crops/varieties and new environmental conditions such as imposed by climate change (Rodenburg and Bastiaans 2011) and soil depletion causing a drop in soil fertility (Joel 2000). The significant yield reductions resulting from infection by parasitic weeds causes food insecurity for millions of subsistence farmers and consequently further increases hunger and poverty

Fig. 3 *Striga hermonthica* infestation of maize field in Kenya

Striga hermonthica: A cereal killer

Origin and distribution

Striga hermonthica (Del.) Benth, commonly known as purple witch weed, is an obligate, out-crossing, hemi-parasitic weed that parasitizes the root systems of its host. It is characterized by small, bright pink flowers. This plant belongs to the genus *Striga* and the family Orobanchaceae (formerly: Scrophulariaceae). Like its main host sorghum, it is considered to originate from the Sudano-Ethiopian region of Africa (Wolfe et al. 2005; Parker 2009). It is most common on heavy soils particularly in the densely populated parts of the Lake Victoria region of Western Kenya (Frost 1994). It infects important cereal crops such as maize, sorghum, pearl millet, finger millet and upland rice, causing devastating losses in yields in sub-Saharan Africa, thereby limiting food supply in many developing countries (Joel 2000; Scholes and Press 2008).

Physiological effects of *Striga*

Upon infection, *Striga* damages the host plant in a number of different ways. Extraction of host-plant photosynthates, water, mineral nutrients and amino acids results in stunted growth and poor yields (Graves et al. 1989; Graves et al. 1990; Musselman 1980; Nickrent and Musselman 2004; Pageau et al. 1998; Press and Gurney 2000). In addition to this a strong 'toxic' or 'pathological' effect, possibly caused by hormonal imbalance in the host, is reported. Due to its infection, an increase in the levels of abscisic acid and reduction in the levels of cytokinins and gibberellic acid was noted that ultimately affects host photosynthesis and growth rate negatively (Gurney et al. 1995; Smith et al. 1995; Taylor et al. 1996; Watling and Press 2001). Moreover, *Striga* infection imposes drought stress on the host due to its high transpiration rate (Ackroyd and Graves 1997; Gebremedhin et al. 2000; Shah et al. 1987). The extent of the actual yield loss is a result of the incidence and severity of attack, the host's susceptibility to *Striga* (Rodenburg et al. 2005), environmental factors (edaphic and climatic) and the crop management (Rodenburg et al. 2006a).

Striga life cycle

The tiny *Striga* seeds (150 x 310 µm, 7 µg) lack the reserves for sustained periods of growth after germination and before host attachment (Berner et al. 1995; Matusova and Bouwmeester 2006). For successful host attachment, germination must take place within 3 to 4 mm of the host root since *Striga* radicles can grow only about 2 to 4 mm (Logan and Stewart 1992; Ramaiah et al. 1991). Therefore *Striga* spp. have evolved an adaptation strategy for successful survival in the field. Before *Striga* seeds can germinate they must lose dormancy through conditioning or warm stratification in a warm moist environment which induces sensitivity to the germination stimulants (Joel et al. 1995; Matusova et al. 2004) (Fig. 4). This is a highly sensitive host recognition strategy as it will initiate the germination process only when it is in close proximity of a suitable host (Fig. 4) and avoids its

germination too far from a host root (Bouwmeester et al. 2003; Hirsch et al. 2003; Yoder 1999). If germination occurs, the radicle grows towards the host root (Logan and Stewart 1992). Contact between the tip of the radicle and the host root initiates an attachment process that leads to the formation of a structure called the haustorium. This haustorium is a multifunctional organ acting as physiological bridge that attaches to a host, establishes a xylem and/or phloem connection and helps in the unidirectional flow of resources to the parasite (Estabrook and Yoder 1998; Yoder 1999; 2001; Sun et al. 2007b). Following the establishment of the connection with the host, the parasite will develop a so-called tubercle that helps to accumulate nutrients. Developing *Striga* plants grow underground for 4 to 7 weeks prior to emergence. Much of the damage to the host already occurs at this stage (Butler 1995; Ejeta 2005). At a certain stage it emerges above the soil, forms a chlorophyll rich shoot, flowers and produces tens of thousands seeds after which the life cycle can start again (Fig. 4).

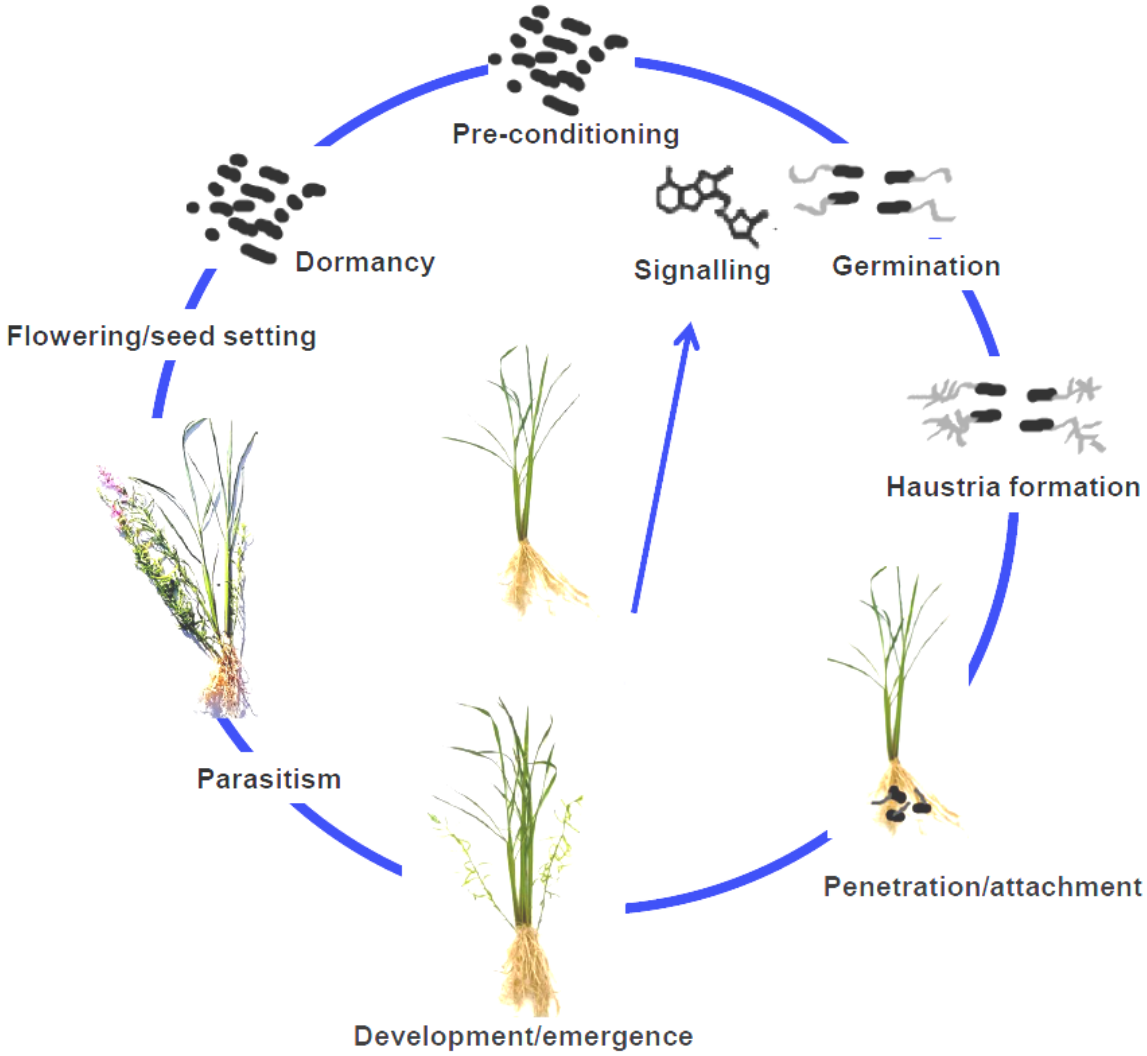

Fig. 4 *Striga hermonthica* life cycle

Strigolactones

About 45 years ago, in 1966, strigol and strigyl acetate were isolated as the first *Striga* germination stimulants from root exudates of the non-host cotton (Cook et al. 1966; Cook et al. 1972) (Table 1; Fig. 5). Strigol was then also identified in the root exudates of sorghum, maize and common millet, all real *Striga* hosts (Siame et al. 1993). The two related germination stimulants, sorgolactone (Hauck et al. 1992) and alectrol (Muller et al. 1992), were isolated from root exudates of sorghum and cowpea, respectively. Brown (1993) defined strigolactones as sesquiterpene lactones. Orobanchol was then isolated from the root exudates of red clover (Yokota et al. 1998; Mori et al. 1999) and it was declared the first *Orobanche* germination stimulant. Furthermore, alectrol and a putative didehydro-orobanchol isomer were also isolated from red clover although alectrol was identified to be orobanchyl acetate recently. In 2005, 5-deoxystrigol was isolated from root exudates of *Lotus japonicus* L. and it was identified that, in addition to their role as germination stimulant, the strigolactones also act as a branching factor for AM fungi (Akiyama et al. 2005). In the same year the strigolactones were renamed as apocarotenoids, when Matusova *et al*. (2005) found that germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. 5-Deoxystrigol was found to be distributed widely in the plant kingdom and was detected in root exudates of monocots (Awad et al. 2006) as well as dicots (Yoneyama et al. 2008). Two exceptional strigolactones, 2'-epi-orobanchol and solanacol (having a phenyl ring) were isolated from root exudates of tobacco (Xie et al. 2007). Similarly in the same period sorgomol was identified in root exudates of sorghum and maize (Awad et al. 2006; Xie et al. 2009b), Chinese milk vetch and white lupin (Yoneyama et al. 2008). 7-Oxo-, 7α-, and 7β-hydroxy orobanchol and their acetates were purified from root exudates of flax (Xie et al. 2009b). Fabacyl acetate (with an epoxide group and opposite ABC rings of strigolactones) was isolated from garden pea (Xie et al. 2009a). In addition to these strigolactones, several novel strigolactones have also been isolated from a variety of plant species but still remain to be identified (Yoneyama et al. 2009a).

Table 1 List of recent identified strigolactones among various plant species

Strigolactones	Plant species	References
strigol	cotton, sorghum, maize, and proso millet	Cook et al. 1966; 1972; Siame et al. 1993; Hauck et al. 1992.
strigyl acetate	cotton	Cook et al. 1966; 1972; Butler 1995.
5-deoxystrigol	*Lotus japonicus*, sorghum, maize, and pearl millet	Akiyama et al. 2005; Awad et al. 2006.
orobanchol	red clover	Yokota et al. 1998.
orobanchyl acetate (alectrol)	cowpea	Müller et al. 1992; Xie et al. 2010.
sorgolactone	sorghum	Hauck et al. 1992.
2'-epi-orobanchol	tobacco	Xie et al. 2007.
solanacol	tobacco	Xie et al. 2007.
sorgomol	sorghum	Awad et al. 2006; Yoneyama et al. 2008.

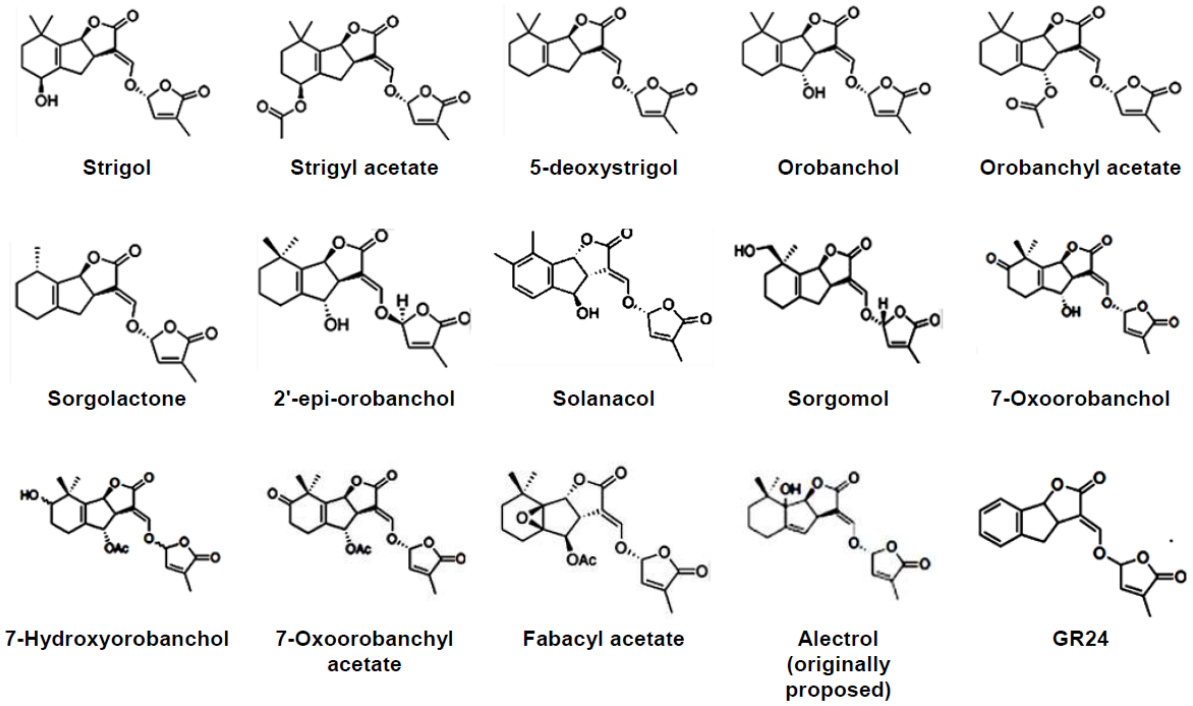

Fig. 5 Structures of natural strigolactones and the synthetic analog GR24, and the structure originally proposed for alectrol (=orobanchyl acetate) (Adapted from Xie et al. 2010).

Biosynthesis and ecological significance

Strigolactones were initially classified as sesquiterpene lactones by many authors (Akiyama et al. 2005) but were reclassified to apocarotenoids when it was shown that the ABC-part of these compounds is derived from the carotenoids likely through the action of a carotenoid cleaving enzyme (Matusova et al. 2005). Indeed it was later demonstrated that Carotenoid Cleavage Dioxygenase 7 (CCD7) and Carotenoid Cleavage Dioxygenase 8 (CCD8), probably catalyzing sequential carotenoid cleavage reactions, are required for the production of strigolactones (Fig. 6) (Gomez-Roldan et al. 2008; Umehara et al. 2008). It is now well documented that significantly less strigolactones are produced due to mutations in *CCD7* or *CCD8* in rice (*dwarf*, high tillering dwarf, *htd*), pea (ramosus, *rms*), and arabidopsis (more axillary growth, *max*) and this causes increased tillering or branching (Umehara et al. 2008; Vogel et al. 2010). By transgenic approaches it was demonstrated that in tomato knocking-down the expression of *CCD7* and *CCD8* leads to reduced strigolactone production and strongly increased branching (Kohlen et al. 2011b; Vogel et al. 2010). Lin et al. (2009) identified *D27*, encoding an iron binding protein in rice, and proved that it is required for strigolactone biosynthesis although they could not establish its enzymatic activity (Fig. 6). Although it was previously hypothesized that one or more cytochrome P450 enzymes are involved in strigolactone biosynthesis (Matusova et al. 2005) so far only one was identified, and only in Arabidopsis (*MAX1*) (Kohlen et al. 2011a). Also for *MAX1*, the catalytic activity of the encoded enzyme is as yet unknown.

Putative orthologs of *MAX1* are present in other plant species, such as rice, but their involvement in strigolactone biosynthesis was so far not proven. In conclusion, the exact biosynthetic pathway of strigolactone biosynthesis is still far from being completely resolved.

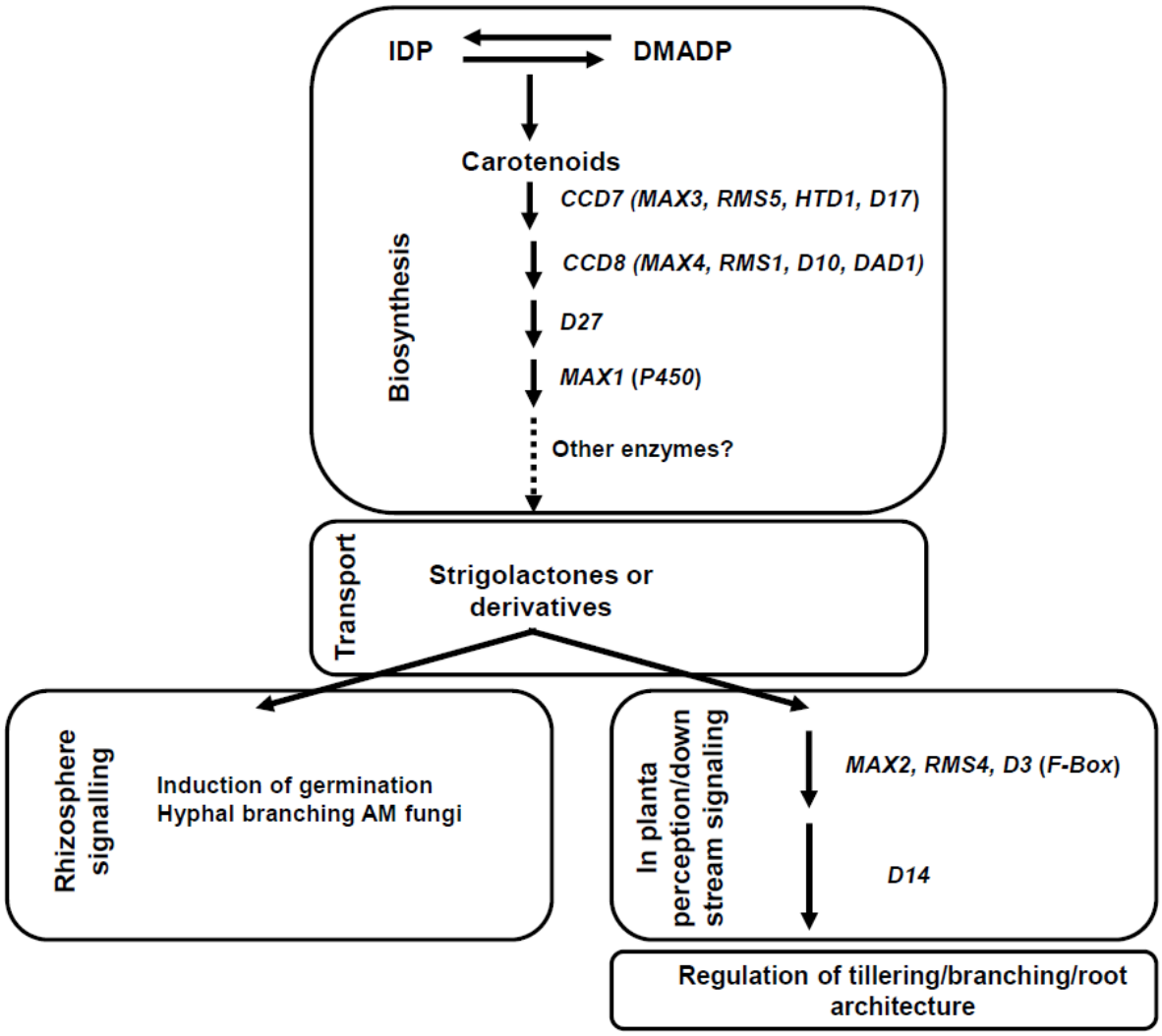

Fig. 6 Schematic diagram showing strigolactone production from carotenoids and their role in the regulation of tillering/branching/root architecture, hyphal branching and germination stimulation. The arrows represent enzymatic steps. IDP, isopentenyl diphosphate; DMADP, dimethylallyl diphosphate; CCD, carotenoid cleavage dioxygenase

Also with regard to the localization of strigolactone biosynthesis much is still unknown. Strigolactones seem to be mainly produced in the roots and from there are either secreted into the rhizosphere or transported upward to the shoot to inhibit shoot branching or tillering. The detection of orobanchol in xylem sap of Arabidopsis and tomato provides evidence that the strigolactones are indeed transported throughout the plant system (Kohlen et al. 2011a). It is thought that 5-deoxystrigol and 2'-epi-5-deoxystrigol are the precursor of all the other strigolactones through hydroxylation, acetylation and/or oxidation (Humphrey and Beale 2006; Matusova et al. 2005; Rani et al. 2008) (Fig. 5). The variation in strigolactone biological activity as germination stimulants, hyphal branching factors or in shoot inhibition might be attributed to differences in their structure (Akiyama et al. 2010;

Garcia-Garrido et al. 2009; Xie et al. 2010; Yoneyama et al. 2009a). Strigolactones are comprised of an ABC part connected to a D-ring through an enol-ether bridge. This enol-ether bridge has been suggested to be mainly responsible for the biological activity of the strigolactones (Akiyama et al. 2010; Yoneyama et al. 2009a; Mangnus and Zwanenburg 1992; Zwanenburg et al. 2009). Nevertheless, small changes, also in the ABC-part of the molecule, do lead to different specific activities of the strigolactones in biological processes in the plant as well as in the rhizosphere.

Striga control

The control of the parasitic *Striga* is much more difficult than of non-parasitic weeds. The production of thousands of seeds by a single plant, the long viability in the soil (>10 years), seed dormancy, and the requirement for germination stimulants from the host are the main factors that hinder the development of successful control measures especially for subsistence farmers (Ejeta et al. 1992; Lagoke et al. 1991; Oryokot 1994). Moreover the weed causes much of the damage to the host during the early below-ground stages of parasitism when infection is not yet evident for the farmer (Eplee and Norris 1995; Parker and Riches 1993). Several control methods have been developed in the past to reduce the parasite population including cultural and mechanical measures (crop rotation, trap and catch cropping, fallowing, hand-pulling, nitrogen fertilization, time and method of planting, intercropping and mixed cropping), physical (solarization), biological and chemical practices (herbicides, synthetic seed germination stimulants, e.g. ethylene or ethephon) and the use of resistant varieties (Berner et al. 1996; Carsky et al. 1994; Gbehounou and Adango 2003; Joel 2000; Kim et al. 1998). However, these control strategies have not been widely practiced due to the mismatch between technologies and the farmers' socio-economic conditions. It has also been suggested that the infection of this parasitic plant can be lowered by reducing strigolactone production in crops (Bouwmeester et al. 2003). As the root parasites exert much of the damage to host crops during the early phases of attachment, *Striga* control strategies that target the pre-attachment stage are attractive. Below we describe a number of approaches that potentially target this pre-attachment stage in the lifecycle of the parasitic weeds.

Carotenoid inhibitors

As described above, the germination stimulants are derived from the carotenoids through cleavage (Matusova et al. 2005). Carotenoid cleavage is a common biosynthetic reaction that occurs in several biosynthetic pathways, including the production of important plant signalling molecules, such as ABA. Considering this biosynthetic origin of the strigolactones, the reduction of strigolactone production in the roots of plants by blocking carotenoid biosynthesis using carotenoid inhibitors could be an interesting approach. In this thesis we investigated whether herbicides that inhibit carotenoid

biosynthesis, such as fluridone, norflurazon and clomazone, can reduce strigolactone production by the host and consequently lower the germination and infection by *Striga*. Since we assume only very low concentrations of these chemical inhibitors are needed, this control strategy may be affordable even to poor small-scale farmers in the African continent.

Pre-attachment resistance

The use of resistant cultivars is considered a cost effective component of integrated *Striga* management (Scholes and Press 2008). As *Striga* germination is dependent upon the quantity and quality of strigolactones (Lopez-Raez et al. 2008; Sun et al. 2007b), genetic variation in this trait could potentially confer pre-attachment resistance (Ejeta 2007). The development of a screening method based on strigolactone-analysis to identify resistant germplasm would benefit breeding efforts targeted at the development of *Striga* resistant cultivars. Combining different resistance mechanisms (pre- and post-attachment resistance for example) into a single cultivar will potentially provide more durable resistance. This can be facilitated by the use of *in vitro* screening methods that allow the dissection of parasitic weed resistance into heritable components (Haussmann et al. 2000; Roman et al. 2002). In this thesis we explored the presence of genetic variation in cereals in strigolactone production and whether this variation correlates with pre-attachment resistance. Hereto, we used MRM-LC-MS, an expensive analytical technique, but we also looked for a correlation between strigolactone production and a more easily detectable phenotype: tillering. This is based on the fact that strigolactones have been identified to play a role in the regulation of above ground plant architecture by inhibiting tillering/shoot branching (Gomez-Roldan et al. 2008; Umehara et al. 2008). Due to this involvement, the tillering phenotype can be assumed to be an indicator of strigolactone production and hence – possibly – of the susceptibility to *Striga* infection. The genetic variation in tillering can then be used as a selection tool to develop cultivars resistant against *Striga* infection.

Soil fertility

In general nitrogen and phosphorus deficiency as well as water stress accentuate the severity of *Striga* damage to the hosts. *Striga* is particularly a pest of low fertile soil and usually the infection decreases if mineral nutrients, especially nitrogen and phosphorus, are applied in sufficient quantities (Adagba et al. 2002; Raju et al. 1990). Obviously, the use of fertilizers not only improves soil fertility but also stimulates the growth and fitness of the host plant and seems to adversely affect germination, attachment and subsequent development of the young *Striga* plants (Cechin and Press 1993; Pieterse and Verkleij 1991). In dicotyledonous plant species there is evidence that the production of strigolactone by the host plant could be reduced if sufficient minerals are available (Lopez-Raez et al. 2008; Yoneyama et al. 2007a). In order to be able to formulate an effective fertilizer strategy for *Striga* control in cereals, it is important to know the exact relationship between fertilizer application

and *Striga* infection. In this thesis we investigated this relationship in a unique combination of lab as well as field studies.

Research objectives

The overall objectives of this PhD study are to determine the relationship between variation in strigolactone production and *Striga* infection in cereals under lab as well as field conditions and based on insights in this relationship, be able to recommend strategies to achieve reduced *Striga* infection in the field.

Outline of the thesis

- Chapter 2 describes the effect of carotenoid inhibitors on strigolactone production and *Striga* seed germination and infection of the host. Hereto, different levels of carotenoid inhibitors were applied to rice either through irrigation or through foliar spray and their effect on strigolactone production and *Striga* infection was evaluated.
- Chapters 3 and 4 describe the genetic variation in pre-attachment resistance and its correlation with strigolactone production. In Chapter 3 a collection of 18 upland rice cultivars called NERICA (New Rice for Africa) and their parents were screened for strigolactone production and *Striga* infection characteristics. The objective of the study was to generate evidence of the existence of pre-attachment resistance in rice based on strigolactones production and to develop a screening method for identification of resistant germplasm.
- In Chapter 4 the correlation between genetic variation in strigolactone production and tillering in a series of rice cultivars collected from all over the world and its effect on *Striga* germination and infection were studied. The objective of this study was to determine whether tillering phenotype of rice could be a suitable selection tool for pre-attachment resistance against *Striga*.
- Chapters 5, 6 and 7 describe *Striga* infection in relation to soil fertility improvement. In Chapters 6 and 7 field trials have been included in parallel with a greenhouse study. Chapter 5 presents an experimental study on quantification of the relationship between strigolactone exudation and *Striga* infection under varying levels of nitrogen and phosphorus.
- Chapter 6 describes the effects of varying nitrogen and phosphorus levels on strigolactone production and consequently on *Striga* infection in maize under greenhouse conditions in the Netherlands and field conditions in Kenya.
- In Chapter 7 the effect of micro-dosing of fertilizer on strigolactone production as well as *Striga* infection is described for three cultivars of sorghum under greenhouse conditions in the Netherlands and field conditions in Mali.
- Finally Chapter 8 presents the general discussion.

Chapter 2

Carotenoid inhibitors reduce strigolactone production and *Striga hermonthica* infection in rice

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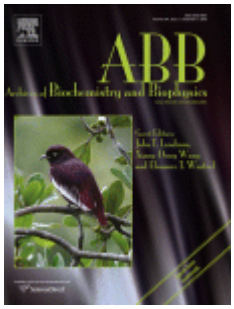

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Abstract

The strigolactones are internal and rhizosphere signalling molecules in plants that are biosynthesized through carotenoid cleavage. They are secreted by host roots into the rhizosphere where they signal host presence to the symbiotic arbuscular mycorrhizal (AM) fungi and the parasitic plants of the *Orobanche*, *Phelipanche* and *Striga* genera. The seeds of these parasitic plants germinate after perceiving these signalling molecules. After attachment to the host root, the parasite negatively affects the host plant by withdrawing water, nutrients and assimilates through a direct connection with the host xylem. In many areas of the world these parasites are a threat to agriculture but so far very limited success has been achieved to minimize losses due to these parasitic weeds. Considering the carotenoid origin of the strigolactones, in the present study we investigated the possibilities to reduce strigolactone production in the roots of plants by blocking carotenoid biosynthesis using carotenoid inhibitors. Hereto the carotenoid inhibitors fluridone, norflurazon, clomazone and amitrole were applied to rice either through irrigation or through foliar spray. Irrigation application of all carotenoid inhibitors and spray application of amitrole significantly decreased strigolactone production, *Striga* germination and *Striga* infection, also in concentrations too low to affect growth and development of the host plant. Hence, we demonstrate that the application of carotenoid inhibitors to plants can affect *Striga* germination and attachment indirectly by reducing the strigolactone concentration in the rhizosphere. This finding is useful for further studies on the relevance of the strigolactones in rhizosphere signalling. Since these inhibitors are available and accessible, they may represent an efficient technology for farmers, including poor subsistence farmers in the African continent, to control these harmful parasitic weeds.

Keywords: carotenoid inhibitors, strigolactone, rice, parasitic plants, *Striga hermonthica*

Introduction

Rice is a cash crop for small to medium scale subsistence farmers of sub-Saharan Africa and during the past few decades is becoming an important and rapidly growing food source throughout the region (Balasubramanian et al. 2007). Due to the increase in the population (4% per annum) and preferences of urban consumers for rice, domestic rice consumption is rising at a rate of 6% per annum (Africa 2007; Rodenburg et al. 2010). To fulfill the demand, Africa is becoming a major importer of rice and its annual share in global import is 32% (FAO 2009). About 9 million tons of rice were imported during 2007, costing about US$ 2 billion per year (Africa 2007). Rice is the 5th cereal in Africa in terms of area harvested with about 9.5 million hectares (ha) during 2008 and has the 4th position in terms of production with about 23 million tons during 2008 (FAO 2009). The average yield of rice in Sub Saharan Africa is very low (2.4 tons ha^{-1}) and constitutes one of the main challenges of rice production (Africa 2007). In addition to other limiting factors, weed competition and especially infestation by parasitic weeds is one of the main causes of low rice yield in the region (Harahap et al. 1993; Johnson et al. 1997; Rodenburg et al. 2010).

Striga is an obligate hemiparasitic plant species that parasitizes grasses and major cereal crops - such as maize, sorghum and millet - in the African continent. It grows on the roots of its host and withdraws photosynthates, minerals and water from the host through a direct connection with the host xylem in an organ called the haustorium. These parasites cause much damage to the host plant and they are a major biotic constraint in cereal production in the African continent imposing a threat to food security (Rodenburg et al. 2010). The yield losses due to infection by *Striga* spp. range from 20 to 80% and severe infestation sometimes leads to complete crop failure (Parker 1991). In sub-Saharan Africa, an estimated 30 to 50 million hectares of cultivated area are infested with seeds of *Striga* spp. and this infestation is increasing day by day contributing to poverty of African people (Emechebe et al. 2004).

Over the past few decades, the global scientific community has done its best to help to find solutions to get rid of this noxious weed and to improve the life of poor African farmers, but so far with only limited success. To date, not even a single efficient and economically viable *Striga* control method is available (Oswald 2005). Integrated *Striga* management has been given due attention in recent years, but the results are limited. The necessity to control *Striga* before emergence, the production of large numbers of *Striga* seeds by even a single plant, the long seed viability and the complicated life cycle of *Striga* spp. are some of the factors responsible for this failure (Ejeta 2007).

Nevertheless, knowledge of the *Striga* lifecycle (Fig. 1) should lay the foundation for *Striga* control. We postulate that control during the earlier stages of the lifecycle may prove most successful instead of at later stages even simply because much of the damage to the host already occurs during the early stages of attachment, prior to *Striga* emergence (Joel 2000). The first interaction between

host and parasite is the induction of germination of the parasite by the host-root secreted germination stimulants (Bouwmeester et al. 2003; Bouwmeester et al. 2007). *Striga* seeds will not germinate without perceiving these signals from the host and these signalling molecules could be a potential target to develop a *Striga* control (Bouwmeester et al. 2003).

Compounds from several different secondary metabolite classes such as dihydrosorgoleone, an isoflavanone, sesquiterpene lactones and strigolactones, have been reported to be secreted by the host root and have germination stimulant activity (Akiyama et al. 2005; Bouwmeester et al. 2003; Bouwmeester et al. 2007).

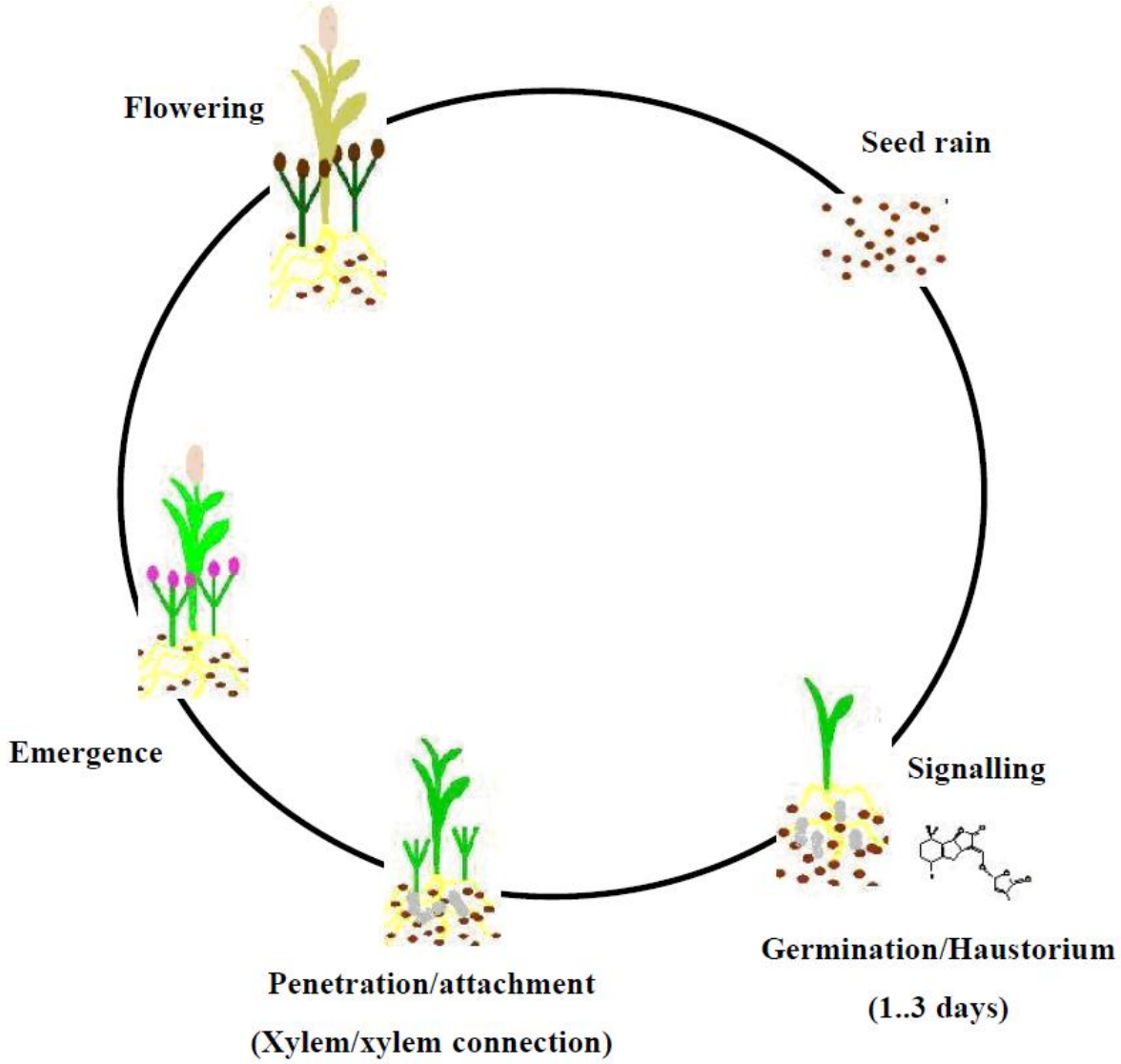

Fig. 1 The life cycle of *Striga hermonthica* (adapted from Sun et al. 2007). After conditioning and perceiving strigolactones, *Striga* seeds can germinate. The radicle grows towards the host root, attaches and penetrates the host root using a specialized feeding structure called haustorium. Developing *Striga* plants grow underground for 4–7 weeks prior to emergence. Much of the damage to the host already occurs at this stage. Then it forms a shoot, emerges above the soil, flowers and produces seeds after which the lifecycle can start again.

However, there is more and more evidence that the strigolactones are the major class of germination stimulants (Bouwmeester et al. 2007). In addition to being host-finding factors for parasitic plants, it was reported some years ago that strigolactones also act as host detection signal for arbuscular mycorrhizal (AM) fungi (Akiyama et al. 2005). Plants can establish a symbiotic relationship with AM fungi in which the fungus helps the plant to take up nutrients from the soil, whereas the plant in return delivers carbohydrates to the fungus. The strigolactones hence have a double role as germination stimulant for parasitic plants and as branching factors for AM fungi (Bouwmeester et al. 2007). Very recently strigolactones were also reported to have an internal, hormonal, signalling function as tillering or shoot branching inhibitors (Gomez-Roldan et al. 2008).

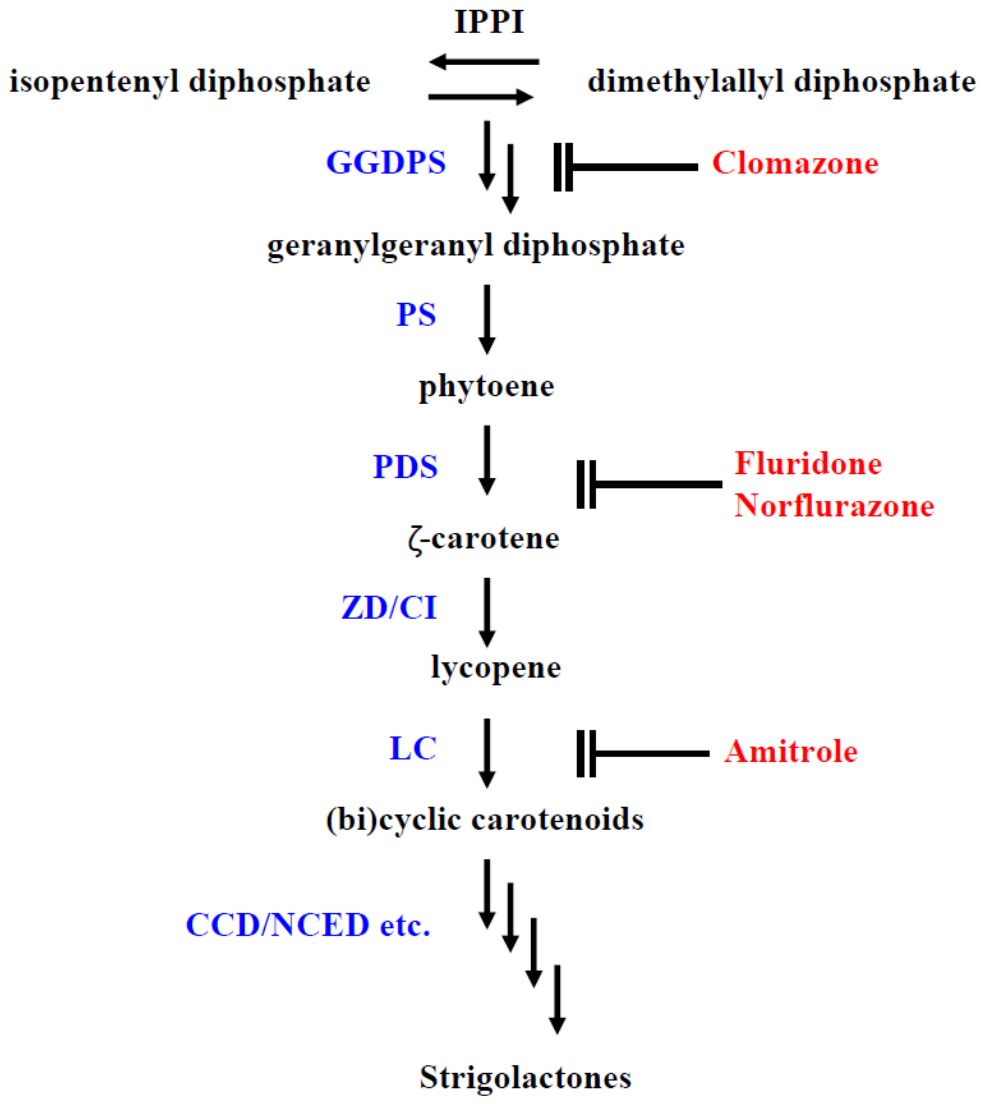

Fig. 2 Schematic diagram showing site of action of various carotenoid inhibitors at various steps in the carotenoid biosynthesis pathway. The arrows represent enzymatic steps. IPPI, isopentenyl diphosphate isomerase; GGDPS, geranylgeranyl diphosphate synthase; PS, phytoene synthase; PDS, phytoene desaturase; ZD, ζ-carotene desaturase; LC, lycopene cyclase; CCD, carotenoid cleavage dioxygenase; NCED, 9-cis-epoxycarotenoid dioxygenase; Fluridone and norflurazon block phytoene desaturase (PDS). Clomazone probably inhibits two key enzymes (GGDPS and IPPI). Amitrole interferes with lycopene cyclase

Quite a few different strigolactones have already been identified in host and some non host plant species (Rani et al. 2008; Xie et al. 2010). Strigol and strigyl acetate were identified in the false host cotton (Hauck et al. 1992; Siame et al. 1993) and strigol was also detected in maize, pearl millet and sorghum (Hauck et al. 1992; Siame et al. 1993). Other strigolactones such as 5-deoxystrigol, sorgolactone and an isomer of strigol, named sorgomol were identified in sorghum (Awad et al. 2006; Yoneyama et al. 2009a). Similarly, orobanchol has been described in red clover, tomato and rice (Akiyama and Hayashi 2006), alectrol in cowpea (Xie et al. 2008) and 2'-epi-5-deoxystrigol and solanacol were recently identified in tobacco (Xie et al. 2007; Yoneyama et al. 2009a). Classically the strigolactones have been described to belong to the sesquiterpene lactones by many authors (Akiyama and Hayashi 2006; Akiyama et al. 2005; Yokota et al. 1998). However, recently it was shown that the strigolactones are derived from the carotenoid biosynthesis pathway (Matusova et al. 2005). Indeed, it was recently demonstrated that two carotenoid cleavage dioxygenases, CCD7 and CCD8, are required for strigolactone biosynthesis (Gomez-Roldan et al. 2008). Hence, as postulated by Matusova et al. (2005) carotenoid cleavage is required for strigolactone biosynthesis, which classifies them as apocarotenoids. In the study by Matusova et al. (2005), the use of the carotenoid inhibitor fluridone was one of the tools to prove the carotenoid origin of the strigolactones. We postulated that this finding may have wider application and hypothesized that the use of carotenoid inhibitors such as fluridone, norflurazon, clomazone and amitrole, in very low concentrations, could be useful as a tool to reduce strigolactone production and ultimately *Striga* seed germination and *Striga* infection (Lopez-Raez et al. 2009). These inhibitors are used - in high concentrations - as herbicides that prevent the formation of carotenoids which leads to photo-bleaching of chlorophyll and hence kills weeds (Böger and Sandmann 1998) (Fig. 2). Fluridone and norflurazon block phytoene desaturase that catalyzes the conversion of phytoene to phytofluene (Breitenbach et al. 2001) (Fig. 2). Clomazone probably inhibits two key enzymes (geranylgeranyl diphosphate synthase and isopentenyl diphosphate isomerase) that control geranylgeranyl diphosphate formation from isopentenyl diphosphate (Boger 1996; Scott et al. 1994). Amitrole has been reported to interfere with lycopene cyclase (Fig. 2) (Stenersen 2004). As described above, inhibition of carotenoid biosynthesis in the shoot will lead to death of the plant as a result of photo-bleaching. Strigolactones, however, are biosynthesized in the roots (Leyser 2008). We postulate that the (mild) inhibition of carotenoid biosynthesis by these inhibitors, particularly in the roots, could lead to a decrease in the production of strigolactones. This may result in decreased germination of *Striga* seeds and hence in reduced *Striga* infection.

Materials and methods

Experimental materials

Four carotenoid inhibitors: fluridone [1-methyl-3-phenyl-5-(3-trifluoromethylphenyl)-4-(1H)-pyridinone], norflurazon [4-chloro-5-methylamino-2-(3-trifluoromethylphenyl) -3(2H)-pyridazinone], clomazone 2-(2-chlorophenyl)-methyl-4,4-dimethyl-3-soxazolidinone] and amitrole (3-amino-l,2,4-triazole) were selected to study their effect on strigolactone production, *Striga* infection and host plant growth. The inhibitors were obtained from Duchefa, the Netherlands (fluridone); Supelco, Germany (norflurazon); Sigma-Aldrich, Germany (clomazone) and Fluka, Germany (amitrole). Spray bottles (Nalgene Aerosol Spray Bottle) were from Cole Parmer, United Kingdom. The synthetic strigolactone, GR24 was obtained from Binne Zwanenburg, Radboud University, Nijmegen, the Netherlands. Standards of orobanchol, 2'-epi-orobanchol, 5-deoxystrigol and 2'-epi-5-deoxystrigol were provided by Koichi Yoneyama, Weed Science Centre, Utsunomiya University, Japan. The mineral nutrients used in Hoagland's nutrient solution (NH_4NO_3, KNO_3, K_2HPO_4, $FeSO_4.7H_2O$, Na_2EDTA, $CaCl_2$, K_2SO_4, $MgSO_4.7H_2O$) were purchased from Merck and Fluka, Germany and Duchefa, the Netherlands. For strigolactone collection SPE C18-Fast columns (500 mg/3 mL) were purchased from Grace Davison Discovery Sciences, Belgium. Organic solvent such as methanol and acetone were from Biosolve, the Netherlands. The mini syringe filters (Minisart SRP_4) were obtained from Sartorius Germany. The seed sterilizing materials as sodium hypochlorite and Tween –20 were purchased from Merck, Germany. The glass fibre filter paper and Whatman filter paper were obtained from Sartorius, Germany and Whatman, United Kingdom, respectively. The seeds of rice cultivar (*cv*) IAC-165 were a gift from Julie Scholes, the University of Sheffield, United Kingdom. *Striga* seeds were a gift from Abdel-Gabar Babiker, Agricultural Research Corporation, Wad Medani, Sudan (collected in 2006 from a sorghum field near Wad Medani) and Cheickna Diarra, Institue d' Economie Rurale (IER) Bamako, Mali (collected in 2000 from a sorghum field near Cinzana, Mali). *Striga* seed viability at the time of experiments was about 60-70% as assessed by a germination assay with 3.3 µM GR24. Germination in water of both batches was negligible.

Experimental details

The experiments were conducted in a completely randomized design (factorial) with three replicates under controlled greenhouse conditions (28°C/25°C with 10h/14h photoperiod and 70% relative humidity) in Wageningen, the Netherlands. The two application methods of carotenoid inhibitors (spray vs irrigation application) were treated as main factors while various levels were tested as sub-factors in each application method. Fluridone, norflurazon and clomazone were applied at three different levels (0.001, 0.01 and 0.05 µM) while amitrole was applied at 0.1, 1.0 and 2.0 µM. Half-strength modified Hoagland's nutrient solution was used as source of minerals nutrients. 250 mL of

this nutrient solution was added to each pot at 2-days intervals up to root exudates collection or *Striga* counting. For irrigation application, carotenoid inhibitors were dissolved in the nutrient solution. In control treatment the nutrient solution was added without any inhibitors. Spray application using a spray bottle was applied to the leaves of rice plants until runoff. Tween-20 (0.02%) was added to each spray mixture as surfactant. In the control treatment, the plants were sprayed with 0.02% Tween-20 in demineralized water.

Strigolactone collection

To analyze strigolactone production, root exudates were collected from each treatment. For this purpose, 10 plants per pot were grown for four weeks using half-strength modified Hoagland's nutrient solution with 100% phosphate. During the 5^{th} week, phosphorus deficiency (10%) was created and different concentrations of the different carotenoid inhibitors were applied to three replicate pots. The pots were treated seven times at 24 h intervals. The carotenoid inhibitors were applied either as irrigation along with nutrient solution or as spray, dissolved in water with 0.02% Tween-20, until run off. In control treatments phosphorus deficient nutrient solution without inhibitors was used (control for irrigation treatment) or a spray with water with 0.02% Tween-20 (control for spray application). Before root exudate collection in the 6^{th} week, the sand in the pots was washed to remove accumulated strigolactones. Hereto, 3 L of nutrient solution containing the respective treatments was applied to the corresponding pots and allowed to drain freely from the pots through holes in the bottom. 48 hours later, root exudates were collected in 1 L plastic bottles from each pot by draining the pots with 1500 mL nutrient solution again containing the respective treatments. The collected root exudates were passed through a C18 column (1.5 g of SPE Bulk Sorbent) and finally the strigolactones were eluted with 6 mL of 100% acetone. The strigolactones, collected in this way, were further studied by LC-MS/MS analysis and *Striga* germination bioassays.

Strigolactone analysis using liquid chromatography-tandem mass spectrometry

The amount of strigolactones in each treatment was quantified through LC-MS/MS analysis. Each sample was concentrated 10-fold using vacuum-centrifugation and cleaned through mini syringe filters (Minisart SRP_4). The retention times and mass transitions of available strigolactone standards such as orobanchol, 2'-epi-orobanchol, 5-deoxystrigol, 2'-epi-5-deoxystrigol, sorgolactone, strigol, solanacol and orobanchyl acetate were compared with each sample to quantify strigolactones using ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) (Lopez-Raez et al. 2008). A Waters Micromass Quattro Premier XE tandem mass spectrometer (Waters, Milford, MA, USA) equipped with an ESI source and coupled to an Acquity UPLC system (Waters, USA) was used. The UPLC was equipped with an Acquity UPLC BEH C_{18} column (150 - 2.1 mm, 1.7 μM) (Waters, USA). Separation was achieved with a water/acetonitrile gradient (starting

at 0% acetonitrile for 0.5 min, raised to 25% acetonitrile in 0.5 min, followed by a 6.5 min gradient to 40% acetonitrile, followed by a 4.5 min gradient to 65% acetonitrile which was then maintained for 0.1 min and followed by a 0.1 min gradient back to 0% acetonitrile before the next run). The column was equilibrated at this solvent composition for 2.05 min. Total run time was 14.25 min. 20 µL samples were injected into the column which was operated at 50°C with a flow-rate of 0.4 mL min^{-1}. For mass spectrometry, positive electro spray ionization (ESI) mode was used. The gas flows for nebulizer and desolvation were 50 and 800 L h^{-1}, respectively. The capillary voltage and cone voltage were 2.7 kV and 20 V, respectively. The source temperature and the desolvation gas temperature were 120°C and 450°C, respectively. For fragmentation, collision-induced dissociation with argon at 3.6 × 10^{-3} mbar was used. To detect strigolactones, multiple reactions monitoring (MRM) was used. The transitions for MRM were set according to the MS/MS spectra obtained for the standards. Protonated molecular ions $[M + H]^+$ were the most abundant in the full-scan mass spectra obtained from the standard strigolactones, therefore, they were selected as parent ions for the transitions. Two or three parent-daughter transitions were selected for each strigolactone, according to the most abundant and/or specific fragment ions for which the collision energy (CE) was optimized. For orobanchol and 2'-epi-orobanchol the transitions (channels) were: *m/z* 347>233 at 10 eV, 347>205 at 15 eV and 347>97 at 18 eV; for 5-deoxystrigol and 2'-epi-5-deoxystrigol the transitions were: *m/z* 331>234, 331>217, 331>97 at the collision energy 10 and 18 eV. MRM transitions *m/z* 361>247 and 361>97 were used for three putative rice strigolactones. Mass Lynx 4.1 software (Waters, USA) was used for data acquisition and analysis. For each compound, the summed peak area of all the corresponding MRM transitions was used for statistical analysis.

***Striga* germination bioassays**

The activity of strigolactones in each treatment was determined using an *in vitro Striga* germination bioassays (Matusova et al. 2005). For pre-conditioning, clean *Striga* seeds were surface sterilized with 2% sodium hypochlorite in sterile water containing 0.4% of Tween-20. Sterilized *Striga* seeds (approximately 50 to100) were placed on 9-mm diameter glass fibre filter paper discs. Twelve of these discs were placed in a 9-cm diameter Petri-dish on sterilized Whatman filter paper, moistened with 3.0 mL sterilized water. After sealing with parafilm, the Petri-dishes were placed in darkness in an incubator for 10 days at 30ºC. After 10 days, the discs with preconditioned *Striga* seeds were placed in a laminar flow cabinet to allow surplus moisture to evaporate. To remove acetone from the strigolactone samples, a mixture of 200 µL of each strigolactone sample (in acetone) and 200 µL of demi water were vacuum centrifuged until the acetone was evaporated. After acetone evaporation, demi water was again added to each sample to make uniform and final volume of 200 µL. Then 50 µL of this sample was applied on triplicate discs in a new Petri dish. A filter paper ring (outer diameter 9 cm, inner diameter 8 cm) moistened with 0.9 mL water was placed in the petri dish to create a humid

atmosphere. GR24 at 3.3 µM was applied as positive control and water as negative control. Subsequently, the *Striga* seeds were incubated in darkness at 30ºC for another 48 hours and germination (seeds with radicle protruding through the seed coat) scored with the help of a binocular microscope (Matusova et al. 2005). To study if carotenoid inhibitors have any direct effect on *Striga* germination, the inhibitors like fluridone, norflurazon, clomazone (0.001, 0.01 & 0.05 µM) and amitrole (0.1, 1.0 & 2.0 µM) were combined with 3.3 µM GR24 and 50 µL of each sample was applied on triplicate discs in a Petri dish.

Striga emergence and attachment

Striga attachment was studied in a pot experiment. Hereto a mixture of 500 mL sand and 20 mg (about 4000) *Striga* seeds was placed in 1.5 L pots. One pre-germinated rice seed *cv* IAC-165 was planted in the middle of each pot. The rice seedlings were allowed to develop in a greenhouse (28°C day (10 h) / 25°C night (14 h) and relative humidity of 70%). About 250 mL half-strength modified Hoagland's nutrient solution was added at 48 hours intervals. One week after planting, carotenoid inhibitors were applied along with the nutrient solution (as irrigation application) or with the spray bottle (dissolved in water with 0.02% Tween-20). The carotenoid inhibitors were applied 12 times (three times a week starting 1 week after planting until five weeks after planting). *Striga* emergence was scored eight weeks after planting. Subsequently, *Striga* attachment was assessed using a binocular microscope, after careful removal of the roots from the sand.

Plant growth and development

Rice growth and development was analysed by measuring plant height, leaf area and plant dry biomass at final harvest.

Statistical analysis

Treatment effects were determined by analyzing data (ANOVA) through GenStat package release 9.2 (PC/Windows XP), VSN international Ltd, UK statistical package. R software package (http://www.r-project.org/) was used for regression analysis to determine the relationships between strigolactones and *Striga* germination. *Striga* attachment (No. per plant) was correlated to the different strigolactones by fitting a Poisson Generalized Linear Mixed Model (GLMM). A stepwise algorithm, based on Akaike Information Criterion (AIC) was used to select the strigolactones that best explain *Striga* germination and attachment for the two different inhibitor application methods (Akaike 1981). The carotenoid inhibitors were taken as random effect and methods as fixed effect.

Results

Effect of carotenoid inhibitors on strigolactone production and rice growth

Irrigation application of fluridone, norflurazon and clomazone significantly reduced the concentration of strigolactones in rice root exudate (Figs 3-5; Table S1). This reduction was significant for the known strigolactones, 2'-epi-5-deoxystrigol and orobanchol but also for the three putative strigolactones (Figs 3-5; Table S1). The effect of irrigation application of amitrole was not significant although there was a negative trend in strigolactone concentration with increasing inhibitor concentration (Fig. 6; Table S1). This was particularly clear for 2'-epi-5-deoxystrigol (Fig. 6A). Maximum reduction in the peak area of the strigolactones was observed with the irrigation application of fluridone, norflurazon and clomazone at 0.05 µM, but even at the lowest concentration of norflurazon that was used (0.001 µM) there was a significant reduction in the peak area of strigolactones (Fig. 4). Spray application of the inhibitors did not have an effect on strigolactones in the root exudate (data not shown) although amitrole application showed a tendency of decreasing the strigolactone concentration (Fig. 7; Table S1).

Rice plant height, leaf area and dry biomass were not affected by the carotenoid inhibitors except for irrigation application of the highest dose of fluridone (0.05 µM) which significantly reduced plant biomass (Table S2). Otherwise, the concentrations of the carotenoid inhibitors used were so small that no effect on growth and development of the host occurred.

Effect of carotenoid inhibitors on *Striga*

The direct application of carotenoid inhibitors in the presence of the synthetic strigolactone analogue GR24 to pre-conditioned *Striga* seeds did not have any negative effect on germination. A mixture of GR24 and the carotenoid inhibitors in the concentrations that were also used to treat the rice plants induced similar germination (60-67%) as GR24 alone (66%) (Table 1). The induction of germination of *Striga* by root exudates of rice was significantly reduced by treatment of the rice plants with carotenoid biosynthesis inhibitors (Table 1). There was a significant negative linear and quadratic relationship between inhibitor concentration and germination for irrigation application of fluridone, clomazone and amitrole (Table 1). For norflurazon the relationship was linear (Table 1). At the highest concentration used (0.05 µM for fluridone, norflurazon and clomazone; 2.0 µM for amitrole) the reduction in *Striga* germination was highest (61-75%) (Table 1). Spray application of the inhibitors did not have a significant effect on the induction of germination by the rice root exudates except for a significant quadratic effect for amitrole. Amitrole spray application at 2.0 µM resulted in a 35% suppression of *Striga* germination (compared with 65% suppression through irrigation application).

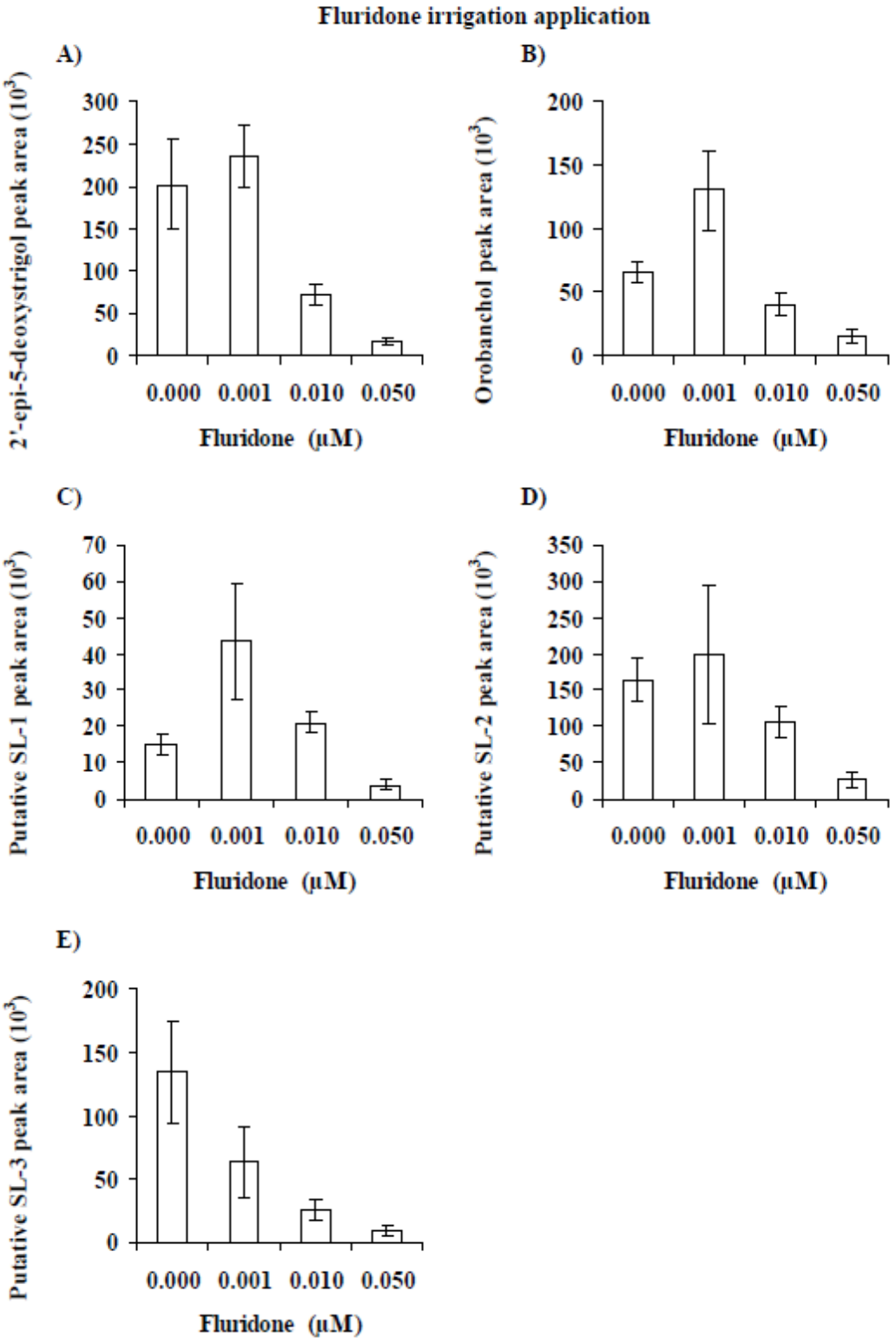

Fig. 3 Inhibition of strigolactone production by fluridone applied through irrigation to rice plants. (A–E) show concentrations of 2'-epi-5-deoxystrigol (A), orobanchol (B) and three putative strigolactones (SL) 1–3 (C–E). Bars represent means of peak areas ± SE of the individual strigolactones as determined by MRM LC–MS in triplicate.

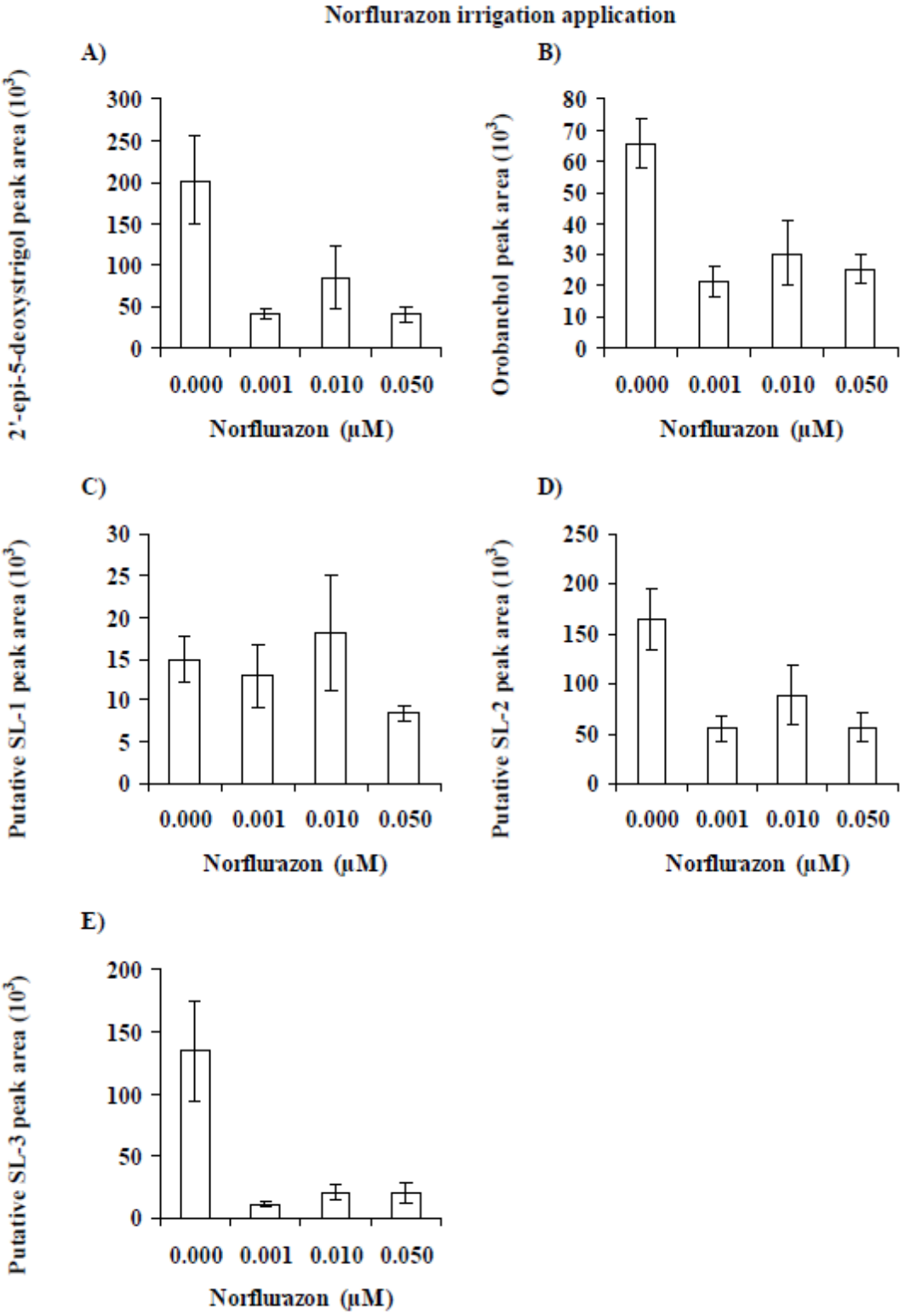

Fig. 4 Inhibition of strigolactone production by norflurazon applied through irrigation. (A–E) show concentrations of 2'-epi-5-deoxystrigol (A), orobanchol (B) and three putative strigolactones (SL) 1–3 (C–E). Bars represent means of peak areas ± SE of the individual strigolactones as determined by MRM LC–MS in triplicate.

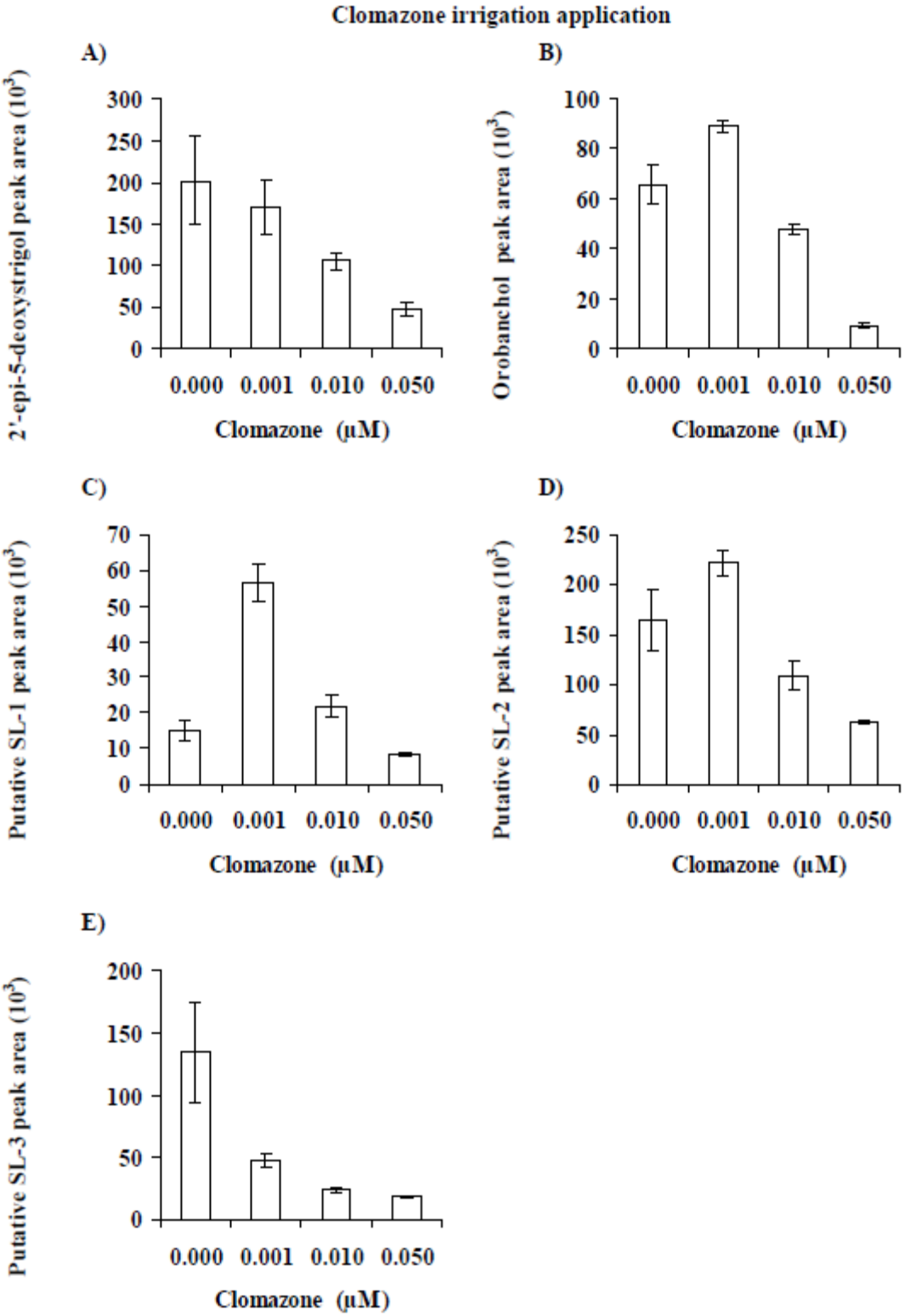

Fig. 5 Inhibition of strigolactone production by clomazone applied through irrigation. (A–E) show concentrations of 2'-epi-5-deoxystrigol (A), orobanchol (B) and three putative strigolactones (SL) 1–3 (C–E). Bars represent means of peak areas ± SE of the individual strigolactones as determined by MRM LC–MS in triplicate

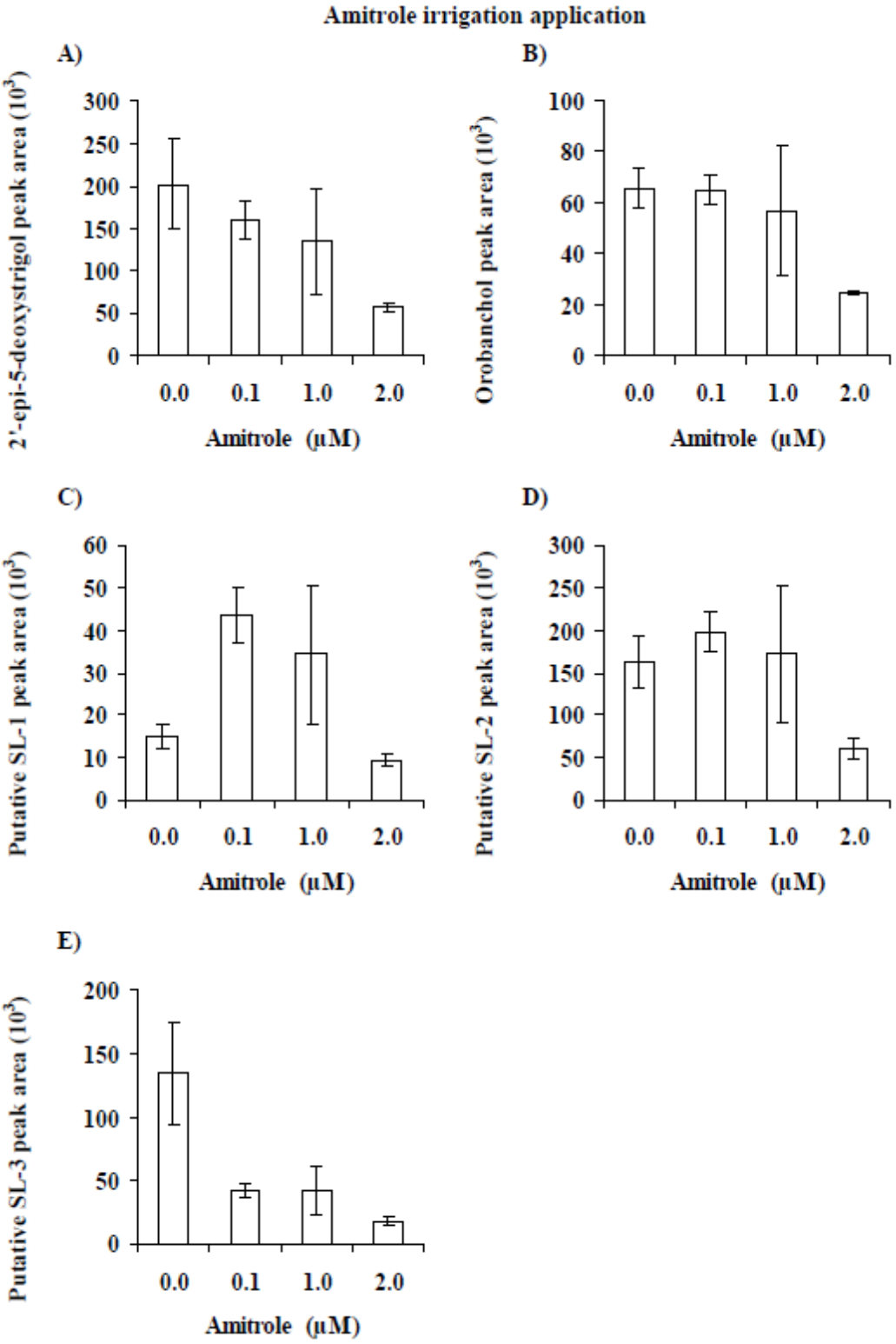

Fig. 6 Inhibition of strigolactone production by amitrole applied through irrigation. (A–E) show concentrations of 2'-epi-5-deoxystrigol (A), orobanchol (B) and three putative strigolactones (SL) 1–3 (C–E). Bars represent means of peak areas ± SE of the individual strigolactones as determined by MRM LC–MS in triplicate.

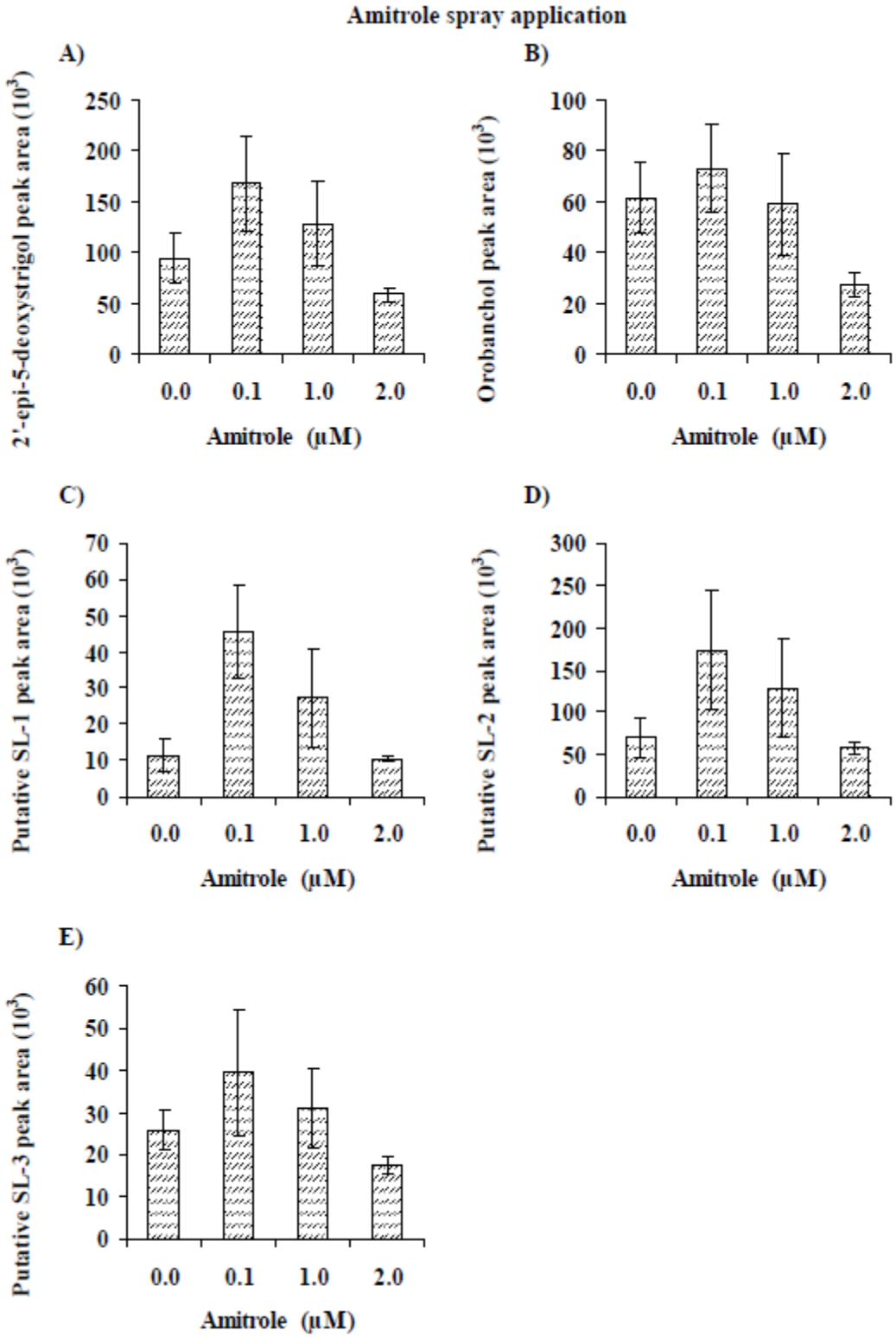

Fig. 7 Inhibition of strigolactone production by amitrole applied through spraying. (A–E) show concentrations of 2'-epi-5-deoxystrigol (A), orobanchol (B) and three putative strigolactones (SL) 1–3 (C–E). Amitrole was dissolved in demineralized water containing 0.02% Tween-20 bars represent means of peak areas ± SE of the individual strigolactones as determined by MRM LC–MS in triplicate

Table 1 Effect of the application of carotenoid inhibitors through irrigation or spray application on the root exudate induced *Striga* germination and *Striga* infection of rice plants growing in pots infected with *Striga* seeds

	Germination (%)		Attachment
	With RE[a]	With GR24[b]	(No. plant^{-1})
A) Fluridone (irrigation application)			
0 µM	57±3 [c **]	66±4 NS[d]	31±6 *
0.001 µM	47±3(-18)	62±4(0)	26±4(-16)
0.01 µM	28±5(-51)	65±4(0)	12±2(-61)
0.05 µM	14±1(-75)	67±4(0)	2±3(-94)
Linear (*P*)	0.001	0.52	0.02
Quadratic (*P*)	0.001	0.99	0.004
LSD (5%)	10.35	-	13.0
B) Norflurazon (irrigation application)			
0 µM	57±3 [a **]	66±4 NS	31±6 *
0.001 µM	21±1(-63)	62±5(-0.1)	17±4(-45)
0.01 µM	19±3(-67)	61±3(-0.1)	20±3(-35)
0.05 µM	22±1(-61)	62±3(-0.1)	10±2(-68)
Linear (*P*)	0.001	0.84	0.02
Quadratic (*P*)	0.29	0.59	0.05
LSD[e] (5%)	12.3	-	12.8
C) Clomazone (irrigation application)			
0 µM	57±3 [a **]	66±4 NS	31±6 *
0.001 µM	53±4(-7)	63±5(0)	26±6(-16)
0.01 µM	33±4(-42)	62±6(-0.1)	18±2(-42)
0.05 µM	18±3(-68)	60±6(-0.1)	11±2(-65)
Linear (*P*)	0.004	0.53	0.08
Quadratic (*P*)	0.001	0.83	0.03
LSD (5%)	11.3	-	14.3
D) Amitrole (irrigation application)			
0 µM	57±3 [a **]	66±4 NS	31±6 NS
0.1 µM	43±4(-25)	61±3(-0.1)	25±5(-19)
1.0 µM	26±5(-54)	65±7(0)	22±5(-29)
2.0 µM	20±2(-65)	67±5(0)	14±3(-55)
Linear (*P*)	0.004	0.58	0.22
Quadratic (*P*)	0.02	0.94	0.14
LSD (5%)	17.5	-	-
E) Amitrole (spray application)			
0 µM	51±2 [a *]	66±4 NS	24±2 NS
0.1 µM	54±9 (+6)	61±3(-0.1)	25±5(+4)
1.0 µM	47±2(-8)	65±7(0)	20±4(-17)
2.0 µM	33±2(-35)	67±5(0)	13±4(-46)
Linear (*P*)	0.67	0.58	0.91
Quadratic (*P*)	0.03	0.94	0.11
LSD (5%)	17.9	-	-

[a] Germination of pre-conditioned *Striga* seeds induced by root exudates collected from rice plant under various carotenoid inhibitors treatments. [b] Germination of pre-conditioned *Striga* seeds induced by GR24, a synthetic strigolactone analogue, mixed with the corresponding concentration of a carotenoid inhibitor.
[c] Means ± standard error (n = 3). [d] Non-significant; figures in parenthesis show percentage decrease (-) or increase (+) compared with the control. [e] Least significant differences of means at P = 0.05 by ANOVA test. * P < 0.05. ** P < 0.01.

Striga attachment and emergence were also influenced by the carotenoid inhibitors. In the irrigation application this effect was significant for fluridone, norflurazon and clomazone but not for amitrole although the trend for amitrole was also a reduction in attachment (Table 1). The highest dose (0.05 µM) of fluridone, norflurazon and clomazone, applied through irrigation, was most effective, causing 65-94% reduction in *Striga* attachment per plant (Table 1). Spray application of carotenoid inhibitors did not have a significant effect on *Striga* attachment.

Correlation between strigolactones and *Striga* infection

Generalized Linear Mixed Model analysis showed that under both application methods, the strigolactones orobanchol and two of the three putative strigolactones contributed significantly to the explanation of the variation in *Striga* germination (Table 2). The variation in attachment obtained in the experiment was significantly explained by the same strigolactones plus putative strigolactone number 3 (Table 2). It was further demonstrated that the strigolactones generally correlated significantly with each other and also germination generally correlated positively with the strigolactones across all treatments (Table S3).

Table 2 Contribution of individual strigolactones to the explanation of the variation in *Striga* germination and attachment

	Germination [a]	Attachment
2'-epi-5-deoxystrigol	NS [b]	NS
orobanchol	**	**
Putative strigolactone 1	**	**
Putative strigolactone 2	**	**
Putative strigolactone 3	NS	**

Linear mixed models were fitted to explain *Striga* germination (after logit transformation) with the individual strigolactones. Generalized linear mixed models with Poisson distribution error and a log link were used to analyze *Striga* attachment with the individual strigolactones (known and unknown). The carotenoid inhibitors were taken as random effect and methods as fixed effect

[a] Germination of pre-conditioned *Striga* seeds in root exudates collected from rice plant under various carotenoid inhibitors treatments; attachment was studied in a pot experiment. See Section ‘‘Materials and methods” for experimental details. [b] Non-significant. ** $P < 0.01$.

Discussion

The strigolactones are host-presence signalling molecules for the parasitic *Striga*, *Orobanche* and *Phelipanche* spp. These parasitic plants require a reliable cue for the presence of a host before they germinate, because they are entirely dependent on a host for survival (Parker 1991). In the present study we show that the biosynthesis of the strigolactones can be inhibited through the use of carotenoid biosynthesis inhibitors. The use of these inhibitors, particularly through irrigation, leads to a decrease in the concentration of strigolactones in rice root exudate, as demonstrated by MRM LC-

MS analysis (Figs 3-7) and germination bioassays (Table 1), and in pot experiments this also leads to reduced *Striga* infection (Table 1).

The recent reclassification of strigolactones from sesquiterpene lactones (Akiyama and Hayashi 2006; Akiyama et al. 2005) to apocarotenoids (Lopez-Raez et al. 2008; Matusova et al. 2005) was based on experiments using inhibitors such as mevastatin, fosmidomycin, fluridone and amitrole that block several different steps in isoprenoid biosynthesis. These inhibitors were applied to individual maize seedlings on nutrient solution. The effect of the treatment was assessed by scoring the induction of germination of parasitic plant seeds by the exudates collected from the treated seedlings. The root exudates of maize treated with fluridone and amitrole induced considerably less germination of the parasitic weed seeds (Matusova et al. 2005). The use of maize carotenoid mutants confirmed these results and this lead to the hypothesis that the ABC-part of the strigolactones is derived from the carotenoids through the action of a carotenoid cleavage enzyme, a carotenoid cleavage dioxygenase (CCD) or a 9-*cis*-epoxycarotenoid dioxygenase (NCED) (Matusova et al. 2005). The present study was carried out to demonstrate whether obstruction of the carotenoid biosynthetic pathway through the use of carotenoid inhibitors can be applied *in planta* to reduce biosynthesis of the strigolactones and thereby *Striga* germination. This would provide a convenient tool in studies on the importance of strigolactones in rhizosphere signalling as well as plant development but, in addition, if this is possible in pot experiments it could potentially also be developed into a parasitic weed control method.

Carotenoid inhibitors are traditionally used as herbicides. They have been shown to inhibit several different enzymatic activities involved in carotenoid biosynthesis in target plant species. The inhibition of carotenoid biosynthesis results in photo-oxidation of the chlorophyll which ultimately leads to the death of the plant (Hess 2000). In the present study the idea was to reduce the amount of carotenoids in the host (non-target plant) with a low and safe concentration of the inhibitors. Since strigolactones are produced in the roots, the inhibition of carotenoid biosynthesis could be restricted to the roots, thereby avoiding photo-bleaching. This could be achieved by the direct application of the inhibitors to the roots through irrigation or by the efficient translocation of inhibitors, after shoot application, to the roots. The inhibition of carotenoid biosynthesis in the roots may reduce germination of parasitic weed seeds as this would lead to less production of signalling molecules, the strigolactones.

Inhibition of phytoene desaturase by fluridone and norflurazon (Breitenbach et al. 2001), geranylgeranyl diphosphate synthase and isopentenyl diphosphate isomerase by clomazone (Scott et al. 1994) and lycopene cyclase by amitrole (Stenersen 2004), all result in decreased carotenoid biosynthesis (Fig. 2). The inhibition of strigolactone formation in the roots of the plant will depend on the absorption and translocation of the carotenoid inhibitors. Fluridone, a systemic and non-selective herbicide, is commonly used to control certain aquatic weeds (Thomas et al. 2002) and has only

limited foliar absorption in terrestrial plants (Berard et al. 1978). Indeed, spray application of fluridone was not effective in reducing the strigolactone concentration in rice root exudate. However, irrigation application of fluridone at 0.01 μM induced a decrease in strigolactone concentration showing that fluridone is taken up by the roots. Norflurazon has been reported to be mobile in the xylem (Mersie and Singh 1987). It is absorbed by plant roots and used to control annual grasses and dicot weeds (Thomas et al. 2002). Application of norflurazon through irrigation was highly effective and strongly reduced strigolactone production even at the lowest rate used (0.001 μM) (Fig. 4) resulting in over 60% lower induction of *Striga* germination by the root exudate (Table 1). Clomazone is translocated in the xylem and taken up by plant roots and it shows very little foliar absorption (Thomas et al. 2002). Indeed, spray application in our study did not have an effect but irrigation application of clomazone in the present study showed fair inhibition of strigolactone biosynthesis, *Striga* germination and *Striga* attachment (Fig. 5; Table 1). Amitrole is considered a non-selective herbicide and is only recommended for use on non-crop plants (Boger 1996; Thomas et al. 2002). However, at the concentrations used in the present study it did not affect rice growth but did lead to a decrease in strigolactone production and a significant decrease in *Striga* germination (Fig. 6, Table 1). Amitrole is a foliar applied herbicide and has been reported to be translocated both by xylem and phloem (Colombia 2004; Thomas et al. 2002). Indeed, amitrole was the only carotenoid inhibitor used in the present study for which spray application also showed tendency of reducing strigolactone production (Fig. 7). This was supported by the significant negative effect of amitrole spray application on *Striga* germination (Table 1).

Striga attachment to the roots of a host plant can only occur after its seed has germinated. *Striga* seed germination requires the presence of strigolactones exuded by the host plant. The reduction in *Striga* germination in *in vitro* germination bioassays with exudates of plants treated with carotenoid inhibitors confirms that carotenoid inhibitors reduce strigolactone production by host plants. We demonstrate here that this reduced germination also leads to reduced *Striga* attachment demonstrating how crucial this first step in the *Striga* lifecycle is for *Striga* but also for control strategies. Statistical analysis showed that particularly orobanchol and the unknown strigolactones contribute to explaining the variation in germination and attachment obtained in our experiments (Table 2). This confirms that the unknown, unidentified strigolactones are really strigolactones and have biological activity. That they are strigolactones is also clear from the fact that they respond to carotenoid inhibitor treatment (Figs 3-7). Their biological activity is supported by results of our on going studies (data not shown) where it has been found that the fractions containing these unknown strigolactones indeed induce germination of *Striga* and branching of AM fungi. In *in vitro* germination bioassays with pure strigolactones, germination of *Striga* was more efficiently induced by 2'-epi-5-deoxystrigol than by orobanchol (data not shown). The significant contribution of orobanchol to the explanation of the variation in germination and attachment in contrast to 2'-epi-5-

deoxystrigol in the present study is therefore perhaps unexpected. However, it is not unlikely that the strong correlation that we found between all the individual strigolactones (because all of them are reduced in a similar fashion by these inhibitors that block very early in the strigolactone pathway) (Table S3) reduces the statistical power to find biological significant correlations.

The present study could be a first step to a new control strategy for *Striga*. Very low doses of carotenoid inhibitors are already effective in inhibiting strigolactone production and lead to decreased *Striga* germination and subsequent infection. Since very low amounts of inhibitors already worked well, this strategy may be economical. In the present study carotenoid inhibitors were applied 12 times to each pot in the *Striga* infection study. Obviously, before application in the field could be attempted, several factors including suitable rate and frequency of application need to be studied. In addition, the present findings were obtained under greenhouse conditions. Before practical application, the effect of sunlight intensity on photo-bleaching needs to be further studied. Photo-bleaching - the loss of photosynthetic pigments - may occur in plants in which carotenoid concentrations have decreased as a result of the application of carotenoid biosynthesis inhibitors. The carotenoids, which are biosynthesized in the chloroplasts, play an essential role in the formation of the light-harvesting and photosynthetic reaction center complexes (Peter and Thornber 1991). They absorb light in certain regions of the visible spectrum, protect chlorophyll by dissolving the oxidative energy of singlet oxygen and dissipate excess light energy in the photosystems. Although the carotenoid levels in plants treated with the lower concentrations of carotenoid inhibitors – that still did reduce strigolactone levels in the root exudate - were not reduced to levels that lead to photo-bleaching it is possible that transition from the relatively low light intensity in the greenhouse to the high light intensity in the field triggers photo-oxidative stress which may lead to photo-bleaching of photosynthetic pigments (Demmigadams and Adams 1992).

Most of the African countries where *Striga* is a problem are also facing droughts and acute water shortage and crop cultivation in most of these areas depends on rainfall (Oyebande 2001). Moreover, the severity of the *Striga* problem has been reported to be greater under water stress in these African rain-fed areas (Boukar et al. 1996). Our results show that particularly irrigation application has a strong effect on strigolactone secretion. Irrigation application is only possible when there is sufficient water available. Nevertheless, spray application of amitrole also showed promising results (Fig. 7). It is clear that the method of application needs further study depending on the local conditions and availability of water. The type of soil can also influence the effectiveness of carotenoid inhibitors. The soil physico-chemical properties as well as molecular structure and formulation can play an important role in adsorption, degradation and movement of the inhibitors. For example, more adsorption of herbicides such as fluridone was reported in silty clay loam soil (Banks and Merkle 1979). Moreover, in general herbicides including fluridone show more efficacy and mobility in

coarse-textured or loamy sand soils (Weber et al. 1986). Our present pot study was carried out in sand and hence the fate of carotenoid inhibitors in other soils such as clay or silt needs to be studied.

Another issue in many of the developing countries where *Striga* is a problem is the cost of pesticides. The income of subsistence farmers in African countries is usually too low to afford costly herbicides, if they are available at all (Verkleij and Kuiper 2000). However, since very low amounts of carotenoid inhibitors were used in the present study, perhaps the use of carotenoid inhibitors to overcome *Striga* infection might prove economical and affordable for the African farmers. As an example we calculated the amount of amitrole required per hectare for one of the treatments (2.0 µM). For irrigation application, if we apply amitrole within the root zone (5 cm depth) to one hectare, we need only about 80 gram *a.i.* of amitrole. This amount should be applied to the soil with about 500,000 liter of water by irrigation. The cost of this amount of amitrole is about € 28,=. Application of carotenoid inhibitors is only possible if enough water is available for irrigation application, or if the carotenoid inhibitors can be applied just before rainfall in rainfed areas. For rainfed areas or upland rice cultivation, spray application may be a better alternative even though in the present experiments spray application was less effective than irrigation application. For spray application of 2.0 µM amitrole, about 160 mg of amitrole *a.i.* in 1000 liter of water is required to treat one hectare. The cost of this amount of amitrole is around €1,= per hectare. It is clear that spray application is an attractive alternative and hence it should be further investigated. New inhibitors that have better foliar absorption or better movement to the roots could be developed and tested.

The choice of a suitable application method, right concentration, timing and number of applications is very important for effective and safe herbicidal weed control (Gitsopoulos and Froud-Williams 2004; Zimdahl 2007). Due to limited access to information and poor literacy, herbicides are often improperly applied. Too late application and/or use of incorrect concentrations may result in inefficient weed control, crop damage and high cost of production (Gitsopoulos and Froud-Williams 2004; Haefele et al. 2000; Johnson et al. 2004). For the purpose of *Striga* control, limited availability of herbicides due to financial constraints or disfunctional markets in many sub-Saharan African countries may pose a serious problem (Balasubramanian et al. 2007).

In conclusion the findings of the present study support the concept that carotenoid biosynthesis inhibitors can be used to reduce the production of strigolactones and their secretion into the rhizosphere which will result in decreased *Striga* germination and consequently lower *Striga* infection. Hence, these carotenoid inhibitors may be used as a tool in studies on the importance of strigolactones in rhizosphere signalling as well as the regulation of plant development. In addition, the effect that the carotenoid biosynthesis inhibitors have on strigolactone secretion and subsequent *Striga* germination and attachment may be developed into a control strategy that might prove efficient, affordable, practical and accessible, even to poor small scale farmers in the African continent.

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Suppl. Table S1 Strigolactone production under irrigation and spray application of varying doses of fluridone, norflurazon, clomazone and amitrole

	2'-epi-5-deoxystrigol	Orobanchol	Putative strigolactones	Strigolactones (over all)	2'-epi-5-deoxystrigol	Orobanchol	Putative strigolactones	Strigolactones (over all)
	(Peak area 10^3)				(Peak area 10^3)			
	A) Fluridone (irrigation application)				**B) Fluridone (spray application)**			
0 µM	201±53 [a*]	66±8 [**]	179±33 [NS]	581±134 [*]	95±25 [*]	62±14 [NS]	108±32 [NS]	265±71 [NS]
0.001 µM	235±36	130±32	244±120	672±153	75±13	40±6	83±21	198±39
0.01 µM	72±13	40±9	128±24	265±50	133±2	57±12	126±33	316±44
0.05 µM	17±5	15±5	30±12	72±26	64±7	37±5	89±27	191±38
Linear (*P*)	0.28	0.01	0.83	0.38	0.88	0.40	0.80	0.76
Quadratic(*P*)	0.001	0.002	0.06	0.006	0.34	0.74	0.70	0.70
LSD (5%)	101.9	45.5	-	379.2	45.2	-	-	-
	C) Norflurazon (irrigation application)				**D) Norflurazon (spray application)**			
0 µM	201±53 [*]	66±8 [*]	179±33 [*]	581±134 [*]	95 ±25 [NS]	62±14 [NS]	108±32 [NS]	265±71 [NS]
0.001 µM	42±7	22±5	68±16	143±27	42±12	22±7	54±8	118±27
0.01 µM	85±36	30±10	107±37	243±87	79±9	41±11	112±11	232±28
0.05 µM	41±10	25±5	64±15	152±37	79±17	43±10	138±21	260±48
Linear (*P*)	0.03	0.01	0.03	0.03	0.10	0.06	0.12	0.09
Quadratic(*P*)	0.15	0.09	0.13	0.12	0.61	0.97	0.11	0.34
LSD (5%)	121.1	27.8	177.2	320.2	-	-	-	-
	E) Clomazone (irrigation application)				**F) Clomazone (spray application)**			
0 µM	201±53 [a *]	66±8 [**]	179±33 [*]	581±134 [*]	95 ±25 [NS]	62 ±14 [NS]	108±32 [*]	265±71 [NS]
0.001 µM	170±34	89±2	278±18	585±58	99±8	56±6	139±8	294±23
0.01 µM	106±10	48±2	130±17	308±31	159±31	80±13	236±42	476±86
0.05 µM	48±8	9±1	72±2	148±9	84±17	43±3	85±5	212±22
Linear (*P*)	0.79	0.001	0.62	0.63	0.41	0.67	0.06	0.21
Quadratic(*P*)	0.01	0.001	0.003	0.003	0.30	0.79	0.18	0.29
LSD (5%)	102.8	15.1	145.5	259.3	-	-	85.0	-
	G) Amitrole (irrigation application)				**H) Amitrole (spray application)**			
0 µM	201±53 [NS]	66±8 [NS]	179±33 [NS]	581±134 [NS]	95±25 [NS]	62±14 [NS]	108±32 [NS]	265±71 [NS]
0.1 µM	160±21	65±6	242±29	509±59	169±47	73±17	258±16	500±173
1.0 µM	134±63	57±26	207±97	440±205	129±42	59±20	186±82	375±143
2.0 µM	57±5	25±1	70±13	169±19	59±6	28±5	86±11	172±22
Linear (*P*)	0.83	0.68	0.85	0.94	0.07	0.30	0.08	0.09
Quadratic(*P*)	0.13	0.16	0.16	0.15	0.24	0.11	0.46	0.32
LSD (5%)	-	-	-	-	-	-	-	-

[*]P <0.05 [**]P <0.01 [***]P <0.001 [NS] Non-significant ; [a.]Means ±standard error (n=3); [LSD]Least significant differences of means at P = 0.05 by ANOVA test.

Suppl. Table S2 Rice plant height, leaf area and total dry biomass under irrigation and spray application of various carotenoid inhibitors

	Plant height (cm)		Leaf area (cm^2)		Total plant dry biomass (g)	
	Irrigation	**Spray**	**Irrigation**	**Spray**	**Irrigation**	**Spray**
Fluridone						
0 µM	107±4 [a**]	100 ±5 [NS]	602 ±11[**]	505±8 [NS]	8.0±0.2[**]	7.5±0.2 [NS]
0.001 µM	108±2	102±1	584±19	480±5	8.0±0.7	7.6±0.3
0.01 µM	102±1	104±2	589±10	435±25	7.1±0.2	7.6±0.2
0.05 µM	53±1	104±1	121±11	503±23	1.0±0.1	7.3±0.6
LSD (5%)	7.5	-	49.3	-	1.4	-
Norflurazon						
0 µM	107 ±4[NS]	100 ±5[NS]	602±11[**]	505±8[NS]	8.0±0.2[NS]	7.5±0.2 [NS]
0.001 µM	106±1	105±2	477±17	490±42	7.6±0.5	7.5±0.2
0.01 µM	108±1	95±0.6	511±21	508±15	7.9±0.3	7.8±0.3
0.05 µM	105±3	101±0.9	594±20	498±2	7.8±0.5	8.3±0.4
LSD (5%)	-	-	68.1	-	-	-
Clomazone						
0 µM	107 ±4[NS]	100±5[NS]	602±11[NS]	505±8[*]	8.0±0.2[NS]	7.5±0.2[NS]
0.001 µM	105±2	105±1	564±39	542±21	7.9±1.4	8.2±0.2
0.01 µM	113±2	103±3	601±53	476±19	8.8±0.2	8.3±0.1
0.05 µM	109±2	99±3	655±16	422±37	7.7±0.4	7.4±0.2
LSD (5%)	-	-	-	69	-	-
Amitrole						
0 µM	107 ±4 [NS]	100±5 [NS]	602±11 [NS]	505±8[*]	8.0±0.2[*]	7.5 ±0.2 [NS]
0.1 µM	111±2	102±1	672±22	417±5	10.1±0.7	6.9±0.3
1.0 µM	117±1	107±2	690±24	466±10	8.6±0.1	7.1±0.1
2.0 µM	111±1	100±2	645±26	385±15	9.7±0.1	7.1±0.2
LSD (5%)	-	-	-	34.9	1.4	-

[*]P <0.05; [**]P <0.01; [NS]: Non-significant [a.]Means±standard error n=3 [LSD]Least significant differences of means at P = 0.05 by ANOVA test.

Suppl. Table S3 Correlations between individual strigolactones and *Striga* germination

	Irrigation application (µM)					Spray application (µM)				
	2'epi5DS	Oro	P SL1	P SL 2	P SL 3	2'epi5DS	Oro	P SL 1	P SL 2	P SL 3
Fluridone										
2'-epi-5-deoxystrigol (2'epi5DS)										
Orobanchol (Oro)	0.96^{**a}					0.67$^{r\,*}$				
Putative strigolactone 1 (P SL 1)	0.70*	0.55^{NS}				0.54^{NS}	0.80**			
Putative strigolactone 2 (P SL 2)	0.68*	0.50^{NS}	0.97**			0.60^{NS}	0.91**	0.86**		
Putative strigolactone 3 (P SL 3)	0.58^{NS}	0.38^{NS}	0.74*	0.88**		0.59^{NS}	0.88**	0.79*	0.98**	
Germination (Germ)	0.94**	0.92**	0.58^{NS}	0.62^{NS}	0.60^{NS}	0.42^{NS}	0.67*	0.60^{NS}	0.73*	0.65^{NS}
Norflurazon										
2'-epi-5-deoxystrigol (2'epi5DS)										
Orobanchol (Oro)	0.90$^{r\,**}$					0.95$^{r\,**}$				
Putative strigolactone 1 (P SL 1)	0.86**	0.82**				0.97**	0.89**			
Putative strigolactone 2 (P SL 2)	0.85**	0.88**	0.92**			0.89**	0.79*	0.94**		
Putative strigolactone 3 (P SL 3)	0.62^{NS}	0.77*	0.54^{NS}	0.81**		0.84**	0.75*	0.88**	0.98**	
Germination (Germ)	0.54^{NS}	0.76*	0.48^{NS}	0.63^{NS}	0.70*	0.70*	0.78*	0.61^{NS}	0.48^{NS}	0.36^{NS}
Clomazone										
2'-epi-5-deoxystrigol (2'epi5DS)										
Orobanchol (Oro)	0.89$^{r\,**}$					0.94$^{r\,**}$				
Putative strigolactone 1 (P SL 1)	0.92**	0.96**				0.84**	0.94**			
Putative strigolactone 2 (P SL 2)	0.90**	0.96**	0.99**			0.90**	0.97**	0.98**		
Putative strigolactone 3 (P SL 3)	0.92**	0.90**	0.98**	0.97**		0.95**	0.88**	0.79*	0.88**	
Germination (Germ)	0.96**	0.94**	0.96**	0.96**	0.94**	0.60^{NS}	0.78*	0.77*	0.75*	0.49^{NS}
Amitrole										
2'-epi-5-deoxystrigol (2'epi5DS)										
Orobanchol (Oro)	0.99$^{r\,**}$					0.99$^{r\,**}$				
Putative strigolactone 1 (P SL 1)	0.97**	0.96**				0.97**	0.94**			
Putative strigolactone 2 (P SL 2)	0.99**	0.98**	0.98**			0.98**	0.97**	0.98**		
Putative strigolactone 3 (P SL 3)	0.97**	0.96**	0.95**	0.98**		0.98**	0.96**	0.99**	0.99**	
Germination (Germ)	0.77**	0.73*	0.78*	0.74*	0.65^{NS}	0.93**	0.89**	0.91**	0.91**	0.93**

[a] $^{*}P$ <0.05; $^{**}P$ <0.01; NS: Non-significant; n=3

Chapter 3

Pre-attachment *Striga hermonthica* resistance of NERICA cultivars based on low strigolactone production

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Abstract

***Striga hermonthica* (*Striga*) is an obligate hemi-parasitic weed, causing severe yield losses in cereals throughout sub-Saharan Africa, including rice. *Striga* germination depends on strigolactones (germination stimulants) exuded by the host roots. The interspecific NERICA (New Rice for Africa) cultivars offer a potentially interesting gene-pool for a screen for low germination inducing rice cultivars. Exudates were collected from all NERICA cultivars and their parents (*O. sativa* and *O. glaberrima*) for analysis of strigolactones. *In-vitro* and *in-situ Striga* germination, attachment, and emergence rates were recorded for each cultivar. NERICA 1 and CG14 produced significantly less strigolactones and showed less *Striga* infection than the other cultivars. NERICAs 7, 8, 11 and 14 produced the highest amounts of strigolactones and showed the most severe *Striga* infection. Across all the cultivars and parents, there was a positive relationship between the amount of strigolactones in the exudate and *Striga* germination, attachment and emergence rates. This study shows that there is genetic variation in *Striga* pre-attachment resistance in NERICA rice. Cultivars combining this pre-attachment resistance with post-attachment resistance (already identified) can provide a key component for durable integrated management of this noxious weed in rice production systems in sub-Saharan Africa.**

Keywords: Strigolactones, *Striga hermonthica* resistance, *Oryza glaberrima*, *Oryza sativa*, NERICA, tillering

Introduction

Rice is an increasingly important staple crop in sub-Saharan Africa (SSA) (Balasubramanian et al. 2007). Over the last three decades the increase in harvested area is about 105% while production increased by 170% (FAO 2009). However, the current domestic production still only covers about 40% of the regional rice consumption (Anonymous. 2007). Africa Rice Center and partners recently developed a collection of inter-specific upland rice cultivars, named NERICA (New Rice for Africa). These cultivars were developed with the aim of combining the high yields from the Asian rice species *O. sativa* (WAB56-104, WAB56-50 and WAB181-18) with the ability of the African species *O. glaberrima* (CG14) to resist local stresses (Jones et al. 1997a, b). To date, 18 interspecific upland cultivars are available to rice farmers. They are popular among rice farmers in the region and are partly responsible for the recent increase in rice area under rain-fed upland conditions (Rodenburg et al. 2006c; Balasubramanian et al. 2007; Wopereis et al. 2008).

However, average rain-fed rice productivity in the region is low as a result of a myriad of production constraints (Balasubramanian et al. 2007) of which weed competition is the most severe (Rodenburg and Johnson 2009). Parasitic plants of the Orobanchaceae such as *Striga* spp. are becoming troublesome weeds in rain-fed rice in sub-Saharan Africa (Rodenburg et al. 2010). Of the *Striga* genus, *Striga hermonthica* (Del.) Benth. is the most damaging species. It is an obligate hemi-parasite, attacking the roots of several cereal crop species leading to severe yield losses (Parker 1991). *Striga* spp. only germinate upon exposure to host-root derived chemicals such as strigolactones (Bouwmeester et al. 2003) which are apocarotenoid signalling molecules (Matusova et al. 2005) released by the host plant into the rhizosphere. After seed germination has been triggered, the radicle of the germinating seed penetrates the host root and forms a haustorium to establish a xylem–xylem connection with the host to withdraw water and nutrients. Subsequently, the parasite emerges above the soil surface, flowers and produces thousands of seeds. Most of the damage to the host occurs between attachment and emergence. Hence, a promising opportunity to minimize losses would be to avoid triggering of *Striga* seed germination, for instance by reducing strigolactones production.

Effective *Striga* control should be based on multiple simultaneously applied strategies. In such an integrated approach, the use of resistant cultivars could be one cost effective element (Scholes and Press 2008). Although genetic variation in *Striga* resistance has been reported in rice in several studies (Harahap et al. 1993; Johnson et al. 1997) only few adapted resistant rice cultivars have been identified to date (Rodenburg et al. 2010). Moreover, in contrast to other host crops such as maize and sorghum, little is known about *Striga* resistance mechanisms in rice. Only two mechanisms have been identified so far: a post-vascular connection resistance (Yoshida and Shirasu 2009) and an incompatibility reaction (Gurney et al. 2006). Both post-attachment resistance mechanisms were found in the rice cultivar Nipponbare which is not adapted to upland rice growing environments.

Existence of pre-attachment resistance, as found in other cereals, is yet to be confirmed in rice. As *Striga* germination is dependent upon the quantity and quality of strigolactone production (Sun et al. 2007; Jamil et al. 2010, 2011a) genetic variation in this trait could potentially confer pre-attachment resistance. One of the reasons for the relatively slow progress in identification of resistant materials and resistance mechanisms is probably the lack of simple and effective screening methods (Ejeta 2007).

The current study aims to determine pre-attachment *Striga* resistance in rice cultivars and to discover if this resistance is based on strigolactone production. To this end, the complete set of 18 upland cultivars of NERICA and their parents were screened for strigolactone production and *Striga* infection characteristics like germination, attachment, emergence and *Striga* dry biomass. The development of a strigolactone-analysis based screening method and identification of resistant germplasm would benefit breeding efforts targeted at the development of *Striga* resistant cultivars. Furthermore, upon confirmation of effectiveness in the field, identification of germplasm with efficient pre-attachment resistance could directly benefit rice farmers in *Striga*-prone areas. This study was carried out in parallel with a study on post-attachment resistance in the NERICAs (Cissoko et al. 2011). Combination of pre- and post-attachment resistance mechanisms against *Striga* spp. into new varieties could lead to more durable resistance against the scourge of SSA.

Materials and methods

Germplasm and chemicals

Seeds of 18 upland NERICA cultivars and their *Oryza sativa* parents WAB56-104, WAB56-50, WAB181-18 and *O. glaberrima* parent CG14 were provided by Africa Rice Center. Seeds of *Striga hermonthica* used in the attachment study were collected from a sorghum field near Cinzana, Mali (courtesy of Cheickna Diarra) and *Striga* seeds used in germination bioassays with rice root exudates were collected from a sorghum field near Wad Medani, Sudan (courtesy of Prof. Abdel Gabar Babiker). *Striga* seed viability was about 60-70%. A standard of orobanchol, was provided by Koichi Yoneyama (Weed Science Center, Utsunomiya University, Japan), 2'-epi-5-deoxystrigol by Kohki Akiyama (Osaka Prefecture University, Japan) and D_6-2'-epi-5-deoxystrigol that was synthesized as described by Ueno *et al.* (2010) and provided by T. Asami (Department of Applied Biological Chemistry, The University of Tokyo, Japan).

Analysis of root exudates

For collection of exudates, 25 germinated seeds of each rice cultivar were planted in a 3 L plastic pot filled with 1.5 L sand. After one week, plants were thinned to 20 plants per pot. Half strength modified Hoagland's nutrient solution with corresponding phosphorus concentration was applied to

each pot (500 mL at 48 hours intervals). The plants were allowed to grow in a climate room (supplemented with artificial lighting 450 µM m^{-2} s^{-1}) under controlled conditions (28°C (D) 10h and 25°C (N) 14h at 70% relative humidity) for four weeks. In the 5th week, phosphorus deficiency was created in each pot to increase strigolactone production (Lopez-Raez et al. 2008). Three L phosphorus deficient nutrient solution (half strength modified Hoagland's nutrient solution minus phosphate) was added to the top of each pot and allowed to drain freely through the holes in the bottom of the pot to remove phosphorus from the sand. The plants were kept under P deficiency for one week. In the 6th week the same draining with 3 L of phosphorus deficient nutrient solution was again added to remove any accumulated strigolactones. Finally, 48 hours later, root exudates were collected in 1 L plastic bottle by passing nutrient solution without phosphate through each pot. The collected root exudates were then run through an SPE C18 column (500 mg per 3 mL) and strigolactones eluted using 6 mL of 100% acetone.

The strigolactones, orobanchol, 2'-epi-5-deoxystrigol and three methoxy-5-deoxystrigol isomers (Fig. 1) (Cardoso et al. unpublished) were identified and quantified using ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) as previously described by Lopez-Raez (2008). The samples were analysed by a Waters Xevo triple quadrupole tandem mass spectrometer (Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) source and coupled to an Acquity UPLC system (Waters, USA). Multiple reactions monitoring (MRM) was used for quantification of strigolactones in rice root exudates. Data acquisition and analysis were performed using Mass Lynx 4.1 (TargetLynx) software (Waters). The biological activity of the exudates of each cultivar was studied using a *Striga* germination bioassay with pre-conditioned *Striga* seeds as described previously (Matusova et al. 2005).

Striga infection

Plastic pots (1.5 L), with a perforated plastic sheet in the bottom, were filled with 100 mL clean, *Striga*-free river sand. On top of this 500 mL of sand mixed with 25 mg (5,000 seeds) *Striga* seeds was added. One pre-germinated seed of each rice cultivar was planted in the middle of each pot and covered by another 100 mL of *Striga*-free sand. Plants were grown in a temperature-controlled glass greenhouse, where natural sun light was supplemented with artificial light (28°C (D) 10 h and 25°C (N) 14h with 70% relative humidity). Half strength modified Hoagland's nutrient solution was applied in the first week (250 ml at 48 h intervals). For the remainder of the experiment a nutrient solution with 20% P was applied to stimulate strigolactone exudation (250 mL to each pot at 48 h intervals). At eight weeks after planting (WAP), rice plants were carefully removed from the sand, the roots carefully washed and *Striga* attachments counted under a stereo microscope.

Striga performance and rice tillering

The third experiment was conducted to determine the final expression of resistance of all cultivars to *Striga* in terms of parasite emergence and biomass production. To this end, 25 mg of *Striga* seeds were thoroughly mixed in 500 mL of a (1:1) potting compost (Lentse Potgrond) / sand mixture which was placed in a plastic pot (1.5 L) with a perforated plastic sheet in the bottom. A pre-germinated seed of each rice cultivar (3 replicates) was planted in the middle of each pot at 2 cm depth and water was applied regularly (250 mL at every 48 hours interval), allowing excess water to drain. The plants were grown in a glass greenhouse, supplemented with artificial lighting under controlled conditions (10h at 28°C (D) and 14h at 25°C (N) at 70% relative humidity). Starting at 4 WAP, *Striga* emergence was recorded weekly from each cultivar for a period of 8 weeks. At 12 WAP, all emerged *Striga* plants were up-rooted, oven-dried at 70^{o}C for 72 hours and weighed to determine total dry biomass of parasite per rice plant. To assess the tillering phenotype, a pre- germinated seed of each cultivar (in 6 replicates) was planted in the middle of a pot containing 500 mL *Striga*-free sand. Nutrient solution with 20% P was applied (250 mL at every 48 hours interval) and the number of tillers per plant counted at 12 WAP.

Statistical analysis

Genstat 9.2 (VSN international Ltd, UK) statistical package was used for ANOVA on *Striga* germination, attachment, emergence and dry biomass data, followed by a Posthoc LSD test for comparison of means. To select the strigolactones that significantly contribute to the explanation of the variation in *Striga* germination, attachment, emergence and dry biomass in the rice cultivars, a stepwise algorithm, based on Akaike Information Criterion (AIC) was used to fit a linear regression model (Akaike 1981). The statistical package *R* was used for correlation analysis to assess how the amounts of different strigolactones correlated to each other. The peak areas of the strigolactones of the NERICAs and their parents were used in redundancy analysis (RDA) using Canoco (ter Braak 1988) to visualize the distance between samples and correlations between explanatory (strigolactones) and response variables (germination, attachment, emergence, dry biomass and tiller numbers).

Results

Strigolactone production

In the UPLC-MS/MS (MRM) chromatograms of the root exudates of the NERICAs and their parents five single intense peaks were detected in the different MRM channels. 2'-epi-5-deoxystrigol was detected at 12.19 min at MRM channels *m/z* 331>234, 331>217 and 331>97, orobanchol was detected at 7.29 min at MRM channels *m/z* 347>233, 347>205, 347>97 and three methoxy-5-deoxystrigol isomers 1-3 were detected at Rt 9.18, 9.78 and 10.33 min at MRM channels *m/z* 361>247 and 361>97

(Figs 1, 2). The same strigolactones were also found in root exudates of the rice cvs IAC 165 and Nipponbare (Jamil et al. 2011c; Cardoso et al. unpublished).

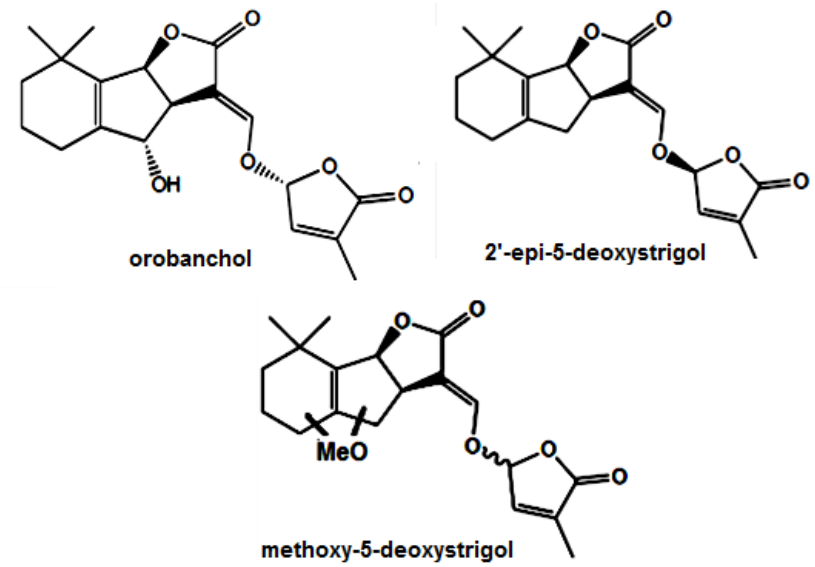

Fig. 1 Chemical structure of orobanchol, 2'-epi-5-deoxystrigol and methoxy-5-deoxystrigol in the root exudates of NEw RICe for Africa (NERICA) cultivars and their parents

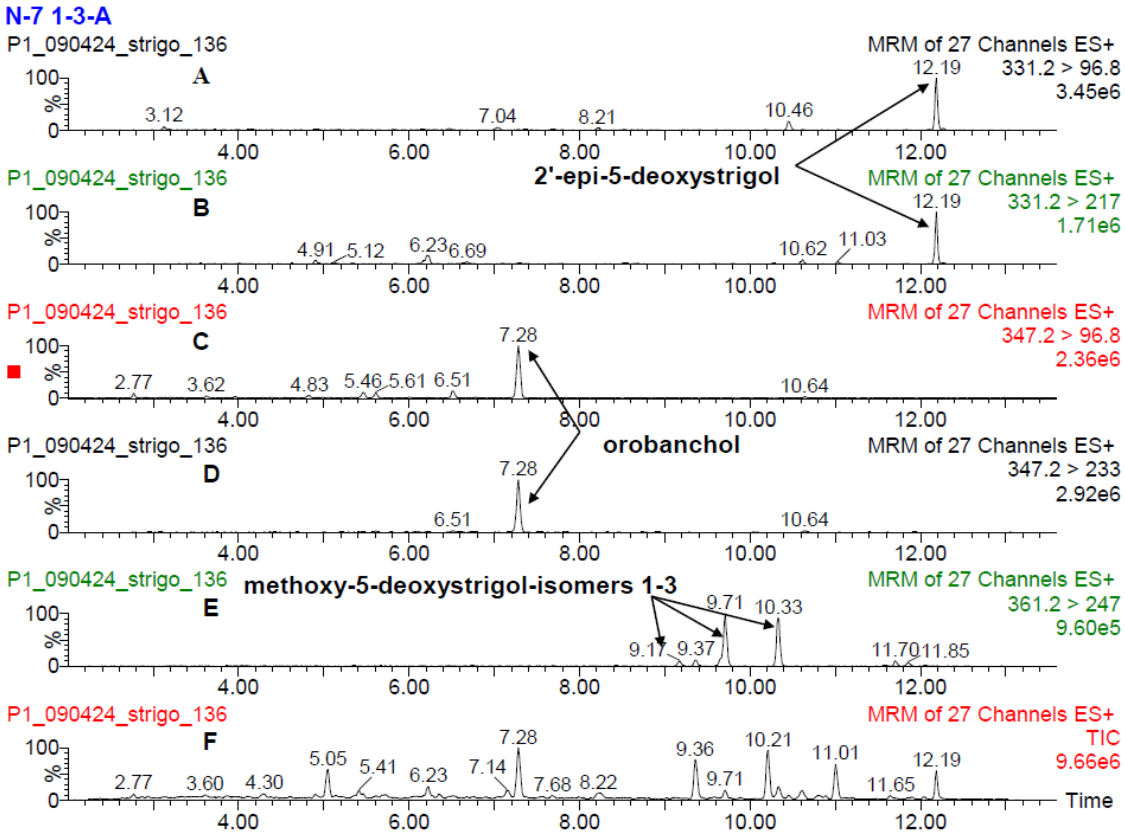

Fig. 2 Liquid chromatography-mass spectrometry (LC-MS) analysis using multiple reaction monitoring (MRM) of rice root exudates. The MRM transitions for 2'-epi-5-deoxystrigol (a, b), orobanchol (c, d), methoxy-5-deoxystrigol isomers 1–3 (e) and total ion current (TIC) (f) obtained for rice *cv* NERICA 7 root exudates are shown as examples

A strong quantitative variation in strigolactone production was observed between the NERICA cultivars (Fig. 3; Suppl. Table S1). WAB56-50 and NERICA cultivars 7, 8, 11 and 14 represented the top five highest strigolactone producers (Fig. 3). NERICA cultivars 6, 10, 15, 2, 9 and 5 showed intermediate production levels, whereas CG14, WAB56-104 and NERICA 1 produced the lowest amount of strigolactones (Fig. 3; Suppl. Table S1). In addition to differences in amount of strigolactones, the cultivars also showed differences in the composition of the strigolactone blend. For example, the proportion of the strigolactones in the total strigolactone blend varied between cultivars from 6.4 to 50.4% for orobanchol, 25.5 to 60.4% for 2'-epi-5-deoxystrigol, 3.5 to 20.9% for methoxy-5-deoxystrigol isomer 2 and 8.8 to 29.8% for methoxy-5-deoxystrigol isomer 3, indicating substantial genetic variation in the strigolactone composition (Fig. 3; Suppl. Table S1).

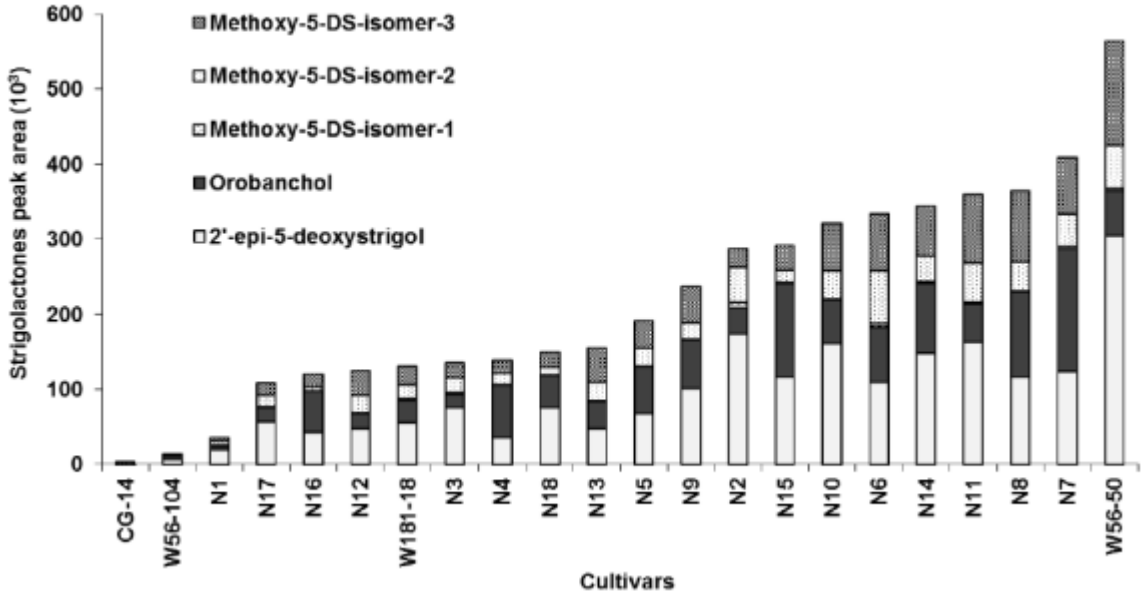

Fig. 3 Production of 2'-epi-5-deoxystrigol, orobanchol and methoxy-5-deoxystrigol (DS) isomers 1–3 by NEw RICe for Africa (NERICA) cultivars and their parents (*Oryza sativa* parents (WAB56-50, WAB56-104, WAB181-18) and *Oryza glaberrima* parent (CG14). The purified root exudates were analysed using multiple reaction monitoring-liquid chromatography-mass spectrometry (MRM-LC-MS) (see the Materials and Methods section). Bars represent means of peak areas of the individual strigolactones as determined by MRM-LC-MS in triplicate

Striga germination, attachment, emergence and dry biomass

Considerable variation was observed in *Striga* germination rates as induced by the root exudates of the different cultivars (Table 1). WAB56-50 and NERICAs 7, 11, 14 and 8 induced the highest *Striga* germination (≥40%), whereas others such as CG14 and NERICA 1 stimulated significantly less *Striga* seed germination than the majority (18) of the other cultivars. The latter two, as well as NERICAs 4 and 3 induced less than 25% *Striga* germination.

Striga attachment was shown to be a less discriminative parameter ($P<0.05$) than *Striga* germination rate ($P<0.001$), but still the top-5 cultivars with the highest germination rates (WAB56-

50 and NERICAs 7, 8, 11 and 14) also had the highest *Striga* attachment (Table 1). On the other hand, of the cultivars showing the lowest germination rates, only CG14 and NERICA 1 also had a similar ranking based on attachment, although the number of attachments on CG14 and NERICA 1 was only significantly different from 20% of the other cultivars. NERICAs 3 and 4, which had low germination rates, had intermediate number of attachments. For most of the intermediate cultivars, the ranking based on attachment was similar to their position based on germination rates.

NERICA 7 had the highest *Striga* emergence, followed by NERICA cultivars 14, 8, 11 and WAB56-50 (>13 plants per pot) (Table 1; Fig. 4). NERICA 1 and *O. glaberrima* parent CG14 had significantly (P<0.01) lower *Striga* emergence than 55% of the other cultivars. The other parent of NERICA 1, WAB56-104 also had a relatively low emergence (ranked 3rd with less than 6 plants per pot) (Table 1; Fig. 4).

In line with the emergence data, WAB56-50 and NERICA 7, 8, 14 and 11 supported the highest *Striga* biomass, while the lowest *Striga* biomass was found on CG14 and NERICA 1 (Table 1). The latter two supported significantly (P<0.01) lower *Striga* biomass than 75% of the other cultivars. NERICA 17, 13, 5 and 12 supported significantly lower *Striga* biomass than 12 of the 14 least performing cultivars (Table 1).

Tiller numbers

Since there is a relationship between strigolactones and tiller numbers (Umehara et al. 2008), the numbers of tillers for the rice cultivars were assessed. Some of the NERICA cultivars such as 6, 7 and 16 had less than 5 tillers per plant while CG14 of the African rice species *O. glaberrima* had as many as 9 tillers per plant (Table 1). The rest of the NERICA cultivars and parents produced between 5 to 7 tillers per plant (Table 1).

Table 1 Tillers per plant and *Striga hermonthica* germination, attachment, emergence and dry biomass (means) for rice cultivars

Cultivars	Rice		*Striga hermonthica*							
	Tillers plant^{-1} (12 WAP)[a]		Germination		Attachment (8 WAP)		Emergence (12 WAP)		Dry biomass (12 WAP)	
Name	Tillers	Rank[b]	%	Rank	No. plant^{-1}	Rank	No. plant^{-1}	Rank	(mg)	Rank
N-1	6.1	8	11.7	2	1.7	2	2.0	1	98	2
N-2	5.6	10	39.5	17	5.7	9	11.7	15	1280	13
N-3	5.1	17	22.9	4	6.3	14	10.0	11	727	8
N-4	5.4	13	16.7	3	6.0	11	10.0	11	1322	14
N-5	5.3	15	26.8	6	5.0	3	8.3	7	419	5
N-6	4.9	20	36.9	15	7.3	17	13.3	17	1402	15
N-7	4.4	22	50.0	21	11.7	22	19.0	22	2255	22
N-8	5.0	19	41.5	18	9.3	20	14.3	19	1750	20
N-9	6.4	6	34.2	11	6.3	15	8.0	6	1506	16
N-10	5.6	10	37.8	16	6.0	12	8.7	8	1554	17
N-11	6.9	2	44.6	20	8.7	19	14.3	19	1694	18
N-12	6.4	6	30.8	9	5.3	7	8.7	8	441	6
N-13	5.6	10	35.8	14	5.7	10	9.0	10	239	4
N-14	5.3	15	42.0	19	7.7	18	16.0	21	1731	19
N-15	5.1	17	35.4	13	6.0	13	12.7	14	1069	11
N-16	4.9	20	27.1	7	5.0	4	7.0	4	1244	12
N-17	6.6	4	28.7	8	5.0	5	7.0	4	235	3
N-18	5.7	9	34.5	12	6.3	16	10.0	11	661	7
W56-104	6.7	3	26.3	5	5.0	6	5.7	3	852	10
W56-50	5.4	13	54.0	22	9.3	21	13.3	18	1839	21
W181-18	6.6	4	32.7	10	5.3	8	12.7	16	793	9
CG14	9.4	1	6.6	1	0.7	1	2.0	1	11	1
P	<0.01		<0.001		<0.05		<0.01		<0.01	
LSD 5%[c]	0.9		11.4		6.0		8.3		603	
SED[d]	0.4		5.7		3.0		4.1		299	

[a]WAP, weeks after planting. [b]Ranking was performed from 'resistant' to 'susceptible', with rank 1 indicating the highest resistance genotype against *Striga* infection and 22 the most susceptible; values are the means of three replicates (for Striga) or six replicates (for Tillers). [c]LSD, Least significant difference of means (5% level); [d]SED, Standard error of difference of means.

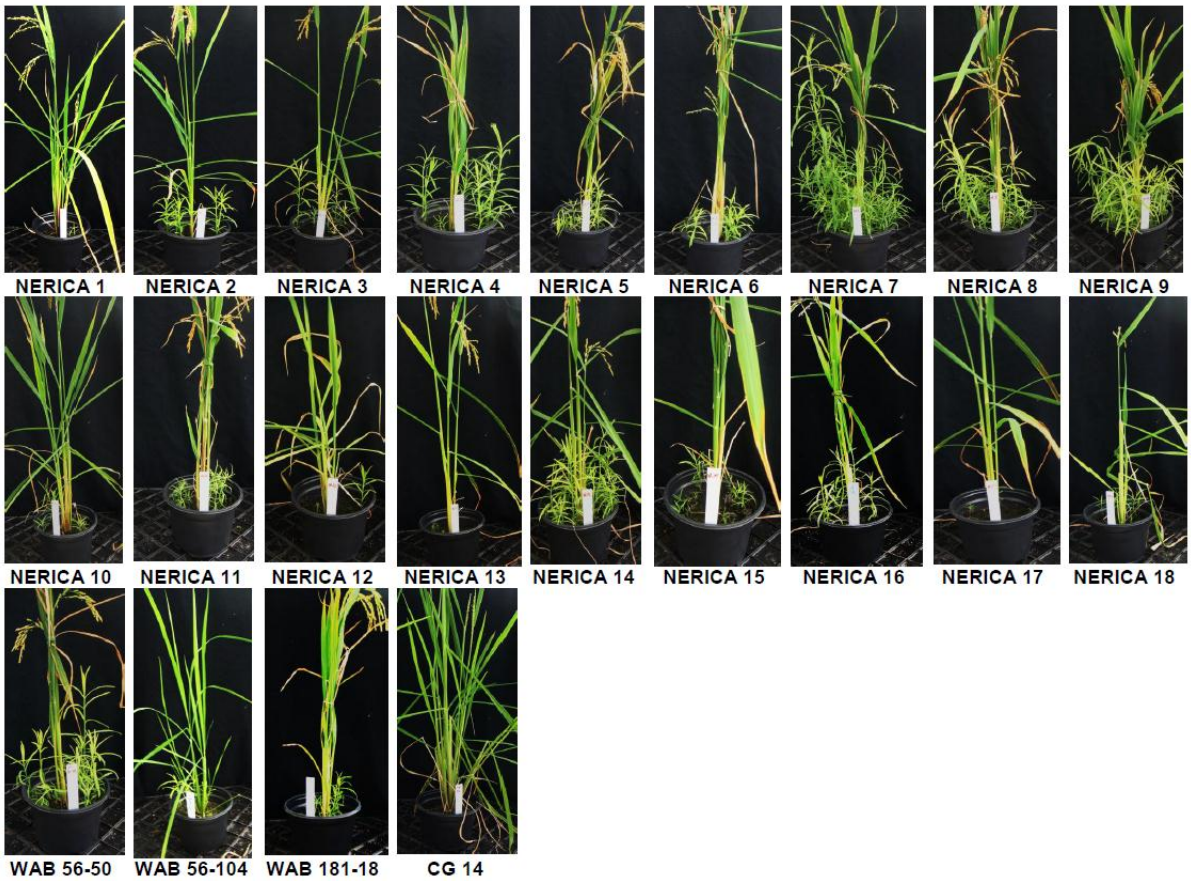

Fig. 4 *Striga* emergence in NEw RICe for Africa (NERICA) cultivars (N1–N18), *Oryza sativa* parents (WAB56-50, WAB56-104, WAB181-18) and *Oryza glaberrima* parent (CG14).

Relationship between strigolactones and *Striga* infection

Striga germination, attachment and emergence positively correlated with the peak areas of 2'-epi-5-deoxystrigol, orobanchol and methoxy-5-deoxystrigol isomers 2 and 3 in the exudates of the NERICAs and their parents (Fig. 5). *Striga* dry biomass positively correlated with the peak area of orobanchol and 2'-epi-5-deoxystrigol in the root exudate.

Methoxy-5-deoxystrigol isomer 1 did not show a significant correlation with any of these parameters. Linear regression showed that the peak areas of 2'-epi-5-deoxystrigol, orobanchol and methoxy-5-deoxystrigol isomer 2 contributed significantly to the explanation of the variation in *Striga* germination induced by the NERICAs and their parents (Table 2). Only orobanchol contributed significantly to the explanation of the variation in *Striga* attachment and emergence, and orobanchol and 2'-epi-5-deoxystrigol showed a significant contribution to the explanation of variation in *Striga* dry biomass (Table 2).

The correlation between the peak areas of the strigolactones was also determined (Suppl. Table S2). Methoxy-5-deoxystrigol isomers 2, 3 and 2'-epi-5-deoxystrigol correlated highly significantly ($P<0.001$) with each other. The correlations between orobanchol, the methoxy-5-deoxystrigol isomers 2, 3 and 2'-epi-5-deoxystrigol were weaker but also significant (Suppl. Table

S2). Methoxy-5-deoxystrigol isomer 1 showed a weaker correlation with most of the other strigolactones, possibly because its concentration was close to the detection level (Suppl. Tables S1 and S2).

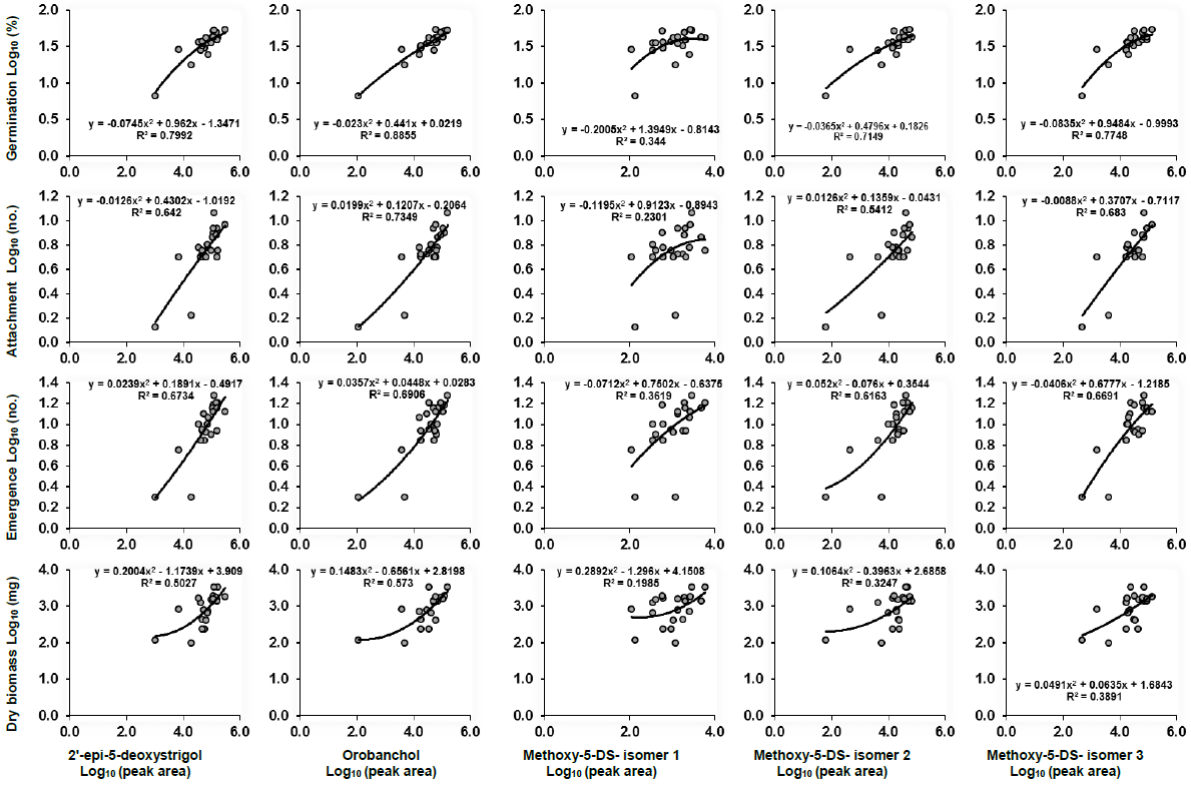

Fig. 5 Relationship between the amounts of 2'-epi-5-deoxystrigol, orobanchol, methoxy-5-deoxystrigol (DS) isomers 1–3 and *Striga* germination, attachment, emergence and dry biomass for all the NEw RICe for Africa (NERICA) cultivars and their parents. The amounts of various strigolactones and *Striga* germination, attachment, emergence and dry biomass were log transformed (log10(x)) and related by correlation analysis.

Table 2 Contribution of strigolactones to the explanation of variation in *Striga hermonthica* germination, attachment and emergence

	Germination	Attachment	Emergence	Dry biomass
2'-epi-5-deoxystrigol	**	NS	NS	*
orobanchol	**	**	**	*
methoxy-5-DS-isomer 1	NS	NS	NS	NS
methoxy-5-DS-isomer 2	**	NS	NS	NS
methoxy-5-DS-isomer 3	NS	NS	NS	NS

$^{*}P$ <0.05; $^{**}P$ <0.01; ^{NS}: Non-significant; The strigolactone peak areas were log transformed [(log(x+0.01)]. Linear regression models were fitted to relate *Striga* germination (logit transformation), attachment and emergence with the best combination of strigolactones

Classification of cultivars

The peak areas of the strigolactones of the NERICAs and their parents were used in redundancy analysis (RDA) to visualize the distance between samples and the correlations between strigolactones (as explanatory variables) and germination, attachment, emergence and tiller numbers (as response variables) (Fig. 6). The scores and loadings biplot shows clustering of NERICA cultivars and their parents based on strigolactone production. The NERICA parent CG14 and NERICA 1, 3, 4, 5 and 17 - all low producers of strigolactones - clustered separately to the right of Principal Component 1 (PC1) while high producers such as WAB56-50 and NERICA 7, 11 and 14 clustered to the left of PC1. The rest of the NERICA cultivars are in between these two groups. The loading plot shows that 2'-epi-5-deoxystrigol, methoxy-5-deoxystrigol isomers 2 and 3 and orobanchol contributed more to the explained variation and separation of samples along PC1 due to larger loading scores on PC1 than methoxy-5-deoxystrigol isomer 1. Orobanchol contributed more to the PC2, explaining variation and separation of samples along PC2 than the other strigolactones. The angle between the arrows in the biplot represents the correlation between the corresponding variables with 0 and 180° indicating the maximum positive and negative correlation and 90° indicating no correlation. Thus, *Striga* germination correlated best with methoxy-5-deoxystrigol isomers 2 and 3 followed by 2'-epi-5-deoxystrigol and methoxy-5-deoxystrigol isomer 1, but less with orobanchol. Methoxy-5-deoxystrigol isomers 1 to 3 also showed positive correlations with attachment, emergence and dry biomass of *Striga* (more than 2'-epi-5-deoxystrigol) whereas orobanchol showed the strongest correlation with these parameters. Similarly, strigolactones correlated negatively with tiller numbers. Of the different strigolactones, orobanchol showed the strongest negative correlation with tiller number. *Striga* attachment, emergence and dry biomass strongly correlated with each other.

Discussion

The present study shows that there is a large variation in pre-attachment *Striga* resistance in the New Rice for Africa (NERICA) collection of rice cultivars derived from crosses between *O. sativa* and *O. glaberrima* species due to differences in strigolactone secretion into the rhizosphere. The method used to screen for pre-attachment resistance is based on UPLC-MS/MS analysis, which enables the quantification and identification of *Striga* seed germination stimulants, the strigolactones, present in the root exudates of *Striga* hosts. The method tested in this study showed to be very accurate for the identification of potentially resistant genotypes.

The first critical step in the life cycle of *Striga*, the germination of its seed, is regulated by the strigolactones (Akiyama and Hayashi 2006; Bouwmeester et al. 2003). It was previously hypothesized that such dependency on host derived signals could be used to the advantage of the crop, for instance

through breeding for low germination stimulant producing cultivars (Bouwmeester et al. 2003; Ejeta 2007; Hess and Ejeta 1992; Lynn and Chang 1990).

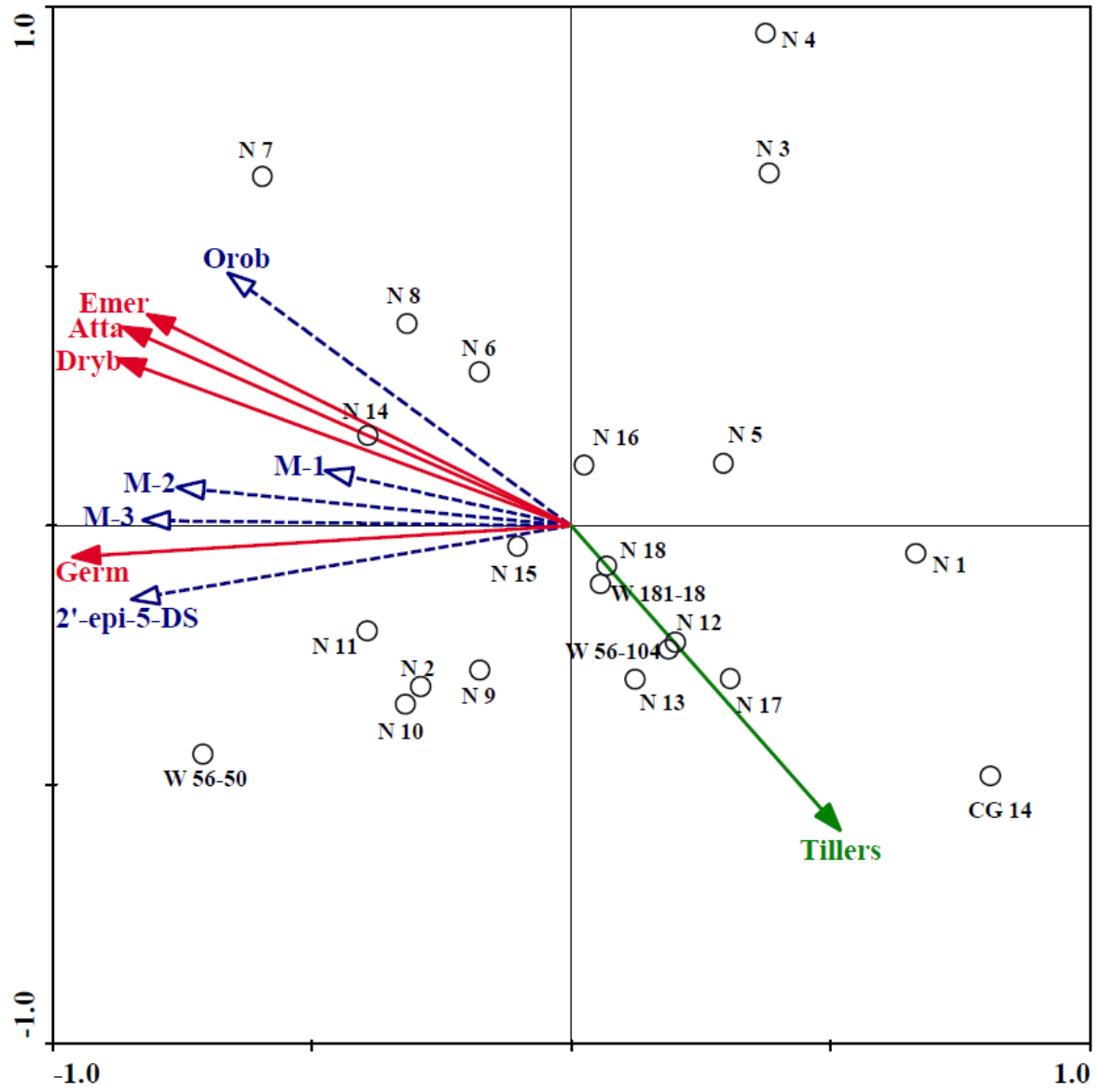

Fig. 6 CANOCO redundancy analysis scores and loadings biplot showing clustering of NEw RICe for Africa (NERICA) cultivars (N1–N18) and their parents *Oryza sativa* (W56-104, W56-50, W181-18) and *Oryza glaberrima* (CG14) based on the amounts of 2'-epi-5-deoxystrigol (2'-epi-5-DS), orobanchol (Orob) and methoxy-5-deoxystrigol isomers 1–3 (M-1, M-2, M-3) as explanatory variables to explain the variation in the response variables *Striga* germination (Germ), attachment (Atta), emergence (Emer), dry biomass (Dryb) and host tillers per plant (Tillers). The explanatory variables (strigolactones) are indicated with dashed arrows and the response variables with solid arrows

Results of the current study, showing significant variation among NERICA cultivars and their parents for strigolactone production and *Striga* germination confirm that such an approach is feasible in rice. The results show that the strigolactone production of a rice cultivar to a large extent explains the level

of resistance against *Striga.* A lower production of strigolactones results in a lower percentage of *Striga* germination, contributing to a more resistant phenotype.

Based on the present findings, breeding for low strigolactone production is likely to result in a certain degree of *Striga* resistance. Previously, significant variation was found for the amount of germination stimulant in sorghum genotypes (Netzly et al. 1988; Rich et al. 2004; Weerasuriya et al. 1993). Sorghum genotypes with low production of the germination stimulant have shown to be resistant to *Striga* in the field (Ejeta 2007; Hess et al. 1992; Ramaiah 1987). Low production of the *Striga* seed germination stimulant in sorghum is inherited as a single recessive gene (Vogler et al. 1996). The high yielding sorghum cultivars containing the low germination stimulant (*lgs*) gene have shown to be very effective against *Striga* and these cultivars have been introduced in many African countries (Ejeta 2005; Ejeta et al. 2000). For rice, only few resistant materials and resistance mechanisms have been identified so far (Rodenburg et al. 2010). The present study confirms the existence of *lgs* as a resistance mechanism in rice cultivars. The methodology tested in the present study showed significant differences in pre-attachment resistance among a group of rice cultivars. Screening of a wider pool of germplasm for the levels of strigolactone production could be helpful for the selection of even more resistant genotypes in the future. The development of cultivars combining resistance with high levels of tolerance (Rodenburg et al. 2006b; Rodenburg et al. 2005) or combining pre- and post-attachment resistance (Cissoko et al. 2011) seems necessary in the near future, if varietal control of *Striga* is to become an important component in integrated management. Such an approach would require screening procedures for individual mechanisms (Rodenburg et al. 2006a; Rodenburg et al. 2005). Post-attachment resistance (Gurney et al. 1995) can for instance complement partial resistance due to low strigolactone production and enhance durability of the resistance, due to the multigenic nature of combined resistance mechanisms.

Arbuscular mycorrhizal fungi (AMF), which are important for providing mineral nutrients to plants through symbiosis, also recognize their host plants through strigolactones (Akiyama et al. 2005; Bouwmeester et al. 2003; Harrison 2005). Hence, symbiosis in low strigolactone producing hosts may be affected negatively. Due attention should, therefore, be paid to the link between strigolactones, *Striga* germination and the colonization by AMF. It might be interesting to test whether AM fungi and *Striga* are triggered by exactly the same type of strigolactones and consequently if it would be an option to identify cultivars that produce the type of strigolactones that stimulate AM fungi without triggering *Striga* germination. In a study by (Cardoso et al. unpublished) activity profiling of rice root exudates with HPLC showed that some fractions induce high *Striga* germination but relatively low AMF branching, while other fractions show much lower germination stimulatory activity and induce high AMF branching. Akiyama and co-workers (Akiyama et al. 2010) confirm that strigolactones such as 5-deoxystrigol and orobanchol are more active in inducing AMF hyphal branching than others such as strigol and sorgomol. By using this knowledge plant breeders could select cultivars that

produce only the desired types of strigolactones that cause maximum AMF hyphal branching but do not induce *Striga* germination.

In addition to quantity, the quality and composition of strigolactones in the root exudates of a host might also be important to explain differences in *Striga* incidence (Netzly et al. 1988; Siame et al. 1993). The differences in significance of correlations between various types of strigolactones and *Striga* infection, as found in the current study, strengthen this assumption (Table 2). Regression analysis shows that 2'-epi-5-deoxystrigol, orobanchol and methoxy-5-deoxystrigol isomer 2 significantly contribute to explain the variation in *Striga* germination (Table 2), whereas Redundancy Analysis suggests that 2'-epi-5-deoxystrigol and methoxy-5-deoxystrigol isomers 2 and 3 are most important for the induction of *Striga* germination (Fig. 6). The latter fits with earlier observations in our lab that the methoxy-5-deoxystrigol isomers are more active stimulants of *Striga* germination than orobanchol (Cardoso et al. unpublished data). Screening for pre-attachment resistance based on strigolactone production should hence focus particularly on finding germplasm with low production of these strigolactones. The fact that there is substantial genetic variation in the composition of the strigolactone blend (Suppl. Table S1; Fig. 3) suggests that this is certainly feasible. Clearly, the biological activities of individual as well as combinations of different strigolactones still need to be studied further to clarify the specific function of individual strigolactones in the rhizosphere.

The negative correlation between tillering and strigolactones and *Striga* infection suggests that higher-tillering cultivars have better *Striga* resistance due to lower strigolactone production. It is remarkable that the arrows for tillering and orobanchol in the biplot almost completely correlate negatively (Fig. 6). Interestingly, orobanchol was recently shown to be present in the xylem of tomato and Arabidopsis, suggesting that it is at least one of the strigolactones involved in the control of shoot branching (Kohlen et al. 2011a). This could suggest that also in rice orobanchol is the strigolactone that regulates tillering, assuming that there is a correlation between the amount of orobanchol in the exudate – which we measured - and in the xylem. Intriguingly, the arrows for attachment, emergence and dry biomass have a slightly different direction from the germination arrow in Fig. 6, and these parameters seem to correlate more with orobanchol than with the other strigolactones. This could be explained by the presence of other resistance mechanisms in NERICA 2, 9, 10, 12, 13 and 17 and WAB56-50 and CG14, that cluster on the low end of PC2, that are absent in NERICA 3, 4 and 7, clustering at the high end of PC2, rather than a causal relation with orobanchol. For some of the former cultivars, (Cissoko et al. 2011) indeed show that they have increased post-attachment resistance. It is hence clear that screening for *Striga* resistance in rice should not only be based on strigolactone production but should also look at post-attachment resistance and expression of these mechanisms in the field. This is predictable as not all of the germinated seeds will establish attachment and also not all of them will emerge from the soil. Clearly, field testing of germplasm selected through screening methods as described here and by Scholes and co-workers (Cissoko et al.

2011), would still be necessary before formulation of recommendations to farmers or breeders. For this field testing, other factors should be closely monitored. For example phosphorus deficiency causes increased production of strigolactones (Lopez-Raez et al. 2008; Yoneyama et al. 2007a; Yoneyama et al. 2007b) and a pre-attachment resistance trait might not be, or not as easily be identified in field screening trials with high phosphorus application. Similarly the *Striga* seed bank density in the soil might affect the apparent pre-attachment resistance. Cultivars with pre-attachment resistance may become ineffective under high *Striga* seed bank density and hence fail to show their resistance phenotype due to heavy soil infestation (Ejeta 2007).

In addition to pre-attachment resistance against *Striga*, associated with low germination stimulant (*lgs*) production, other resistance mechanisms such as low production of the haustorial initiation factor (*lhf*) or an incompatibility response (*ir*) to *Striga* parasitism by the host could act as additional factors in selection of resistant genotypes (Ejeta et al. 2000). For haustorial initiation the parasite requires an additional host signal and on host roots of genotypes possessing *lhf* resistance, germinated parasitic seeds fail to form haustoria and to attach to their potential host and consequently die (Riopel and Timko 1995). Similarly, due to the incompatibility reaction in some genotypes, penetrated *Striga* seedlings show stunted growth and stop further development after first emergence of leaves due to lack of nutrients from the host. The incompatibility reaction mainly occurs as a result of inadequate xylem-xylem connections between host and parasite. Also in rice, post-attachment *Striga* resistance has been identified (Gurney et al. 2006; Yoshida and Shirasu 2009). The symptoms for this are necrosis around the site of parasite attachment and inability of the parasite to penetrate the endodermis. In other plant species also other mechanical barriers such as lignification (Maiti et al. 1984), cellulose accumulation (Olivier et al. 1991) and encapsulation of the parasite (Labrousse et al. 2001) have been found to be responsible for failure of the connection of the parasite's vascular tissue to that of the host. Strigolactone production correlates well with the level of resistance against *Striga*, suggesting that germination is an important component of resistance. However, other parameters affecting the attachment and emergence success further determine whether successful *Striga*-host relations are established or not. It is interesting to note that some of the cultivars that produce low amounts of strigolactones also exhibit good post attachment resistance to several different *Striga* ecotypes (Cissoko et al. 2011).

In conclusion, pre-attachment *Striga* resistance due to low induction of *Striga* germination as a result of low strigolactone production exists in the NERICA collection. This pre-attachment resistance can be determined through identification and quantification of strigolactones present in root exudates by LC/MS analysis. Based on this information, a rice cultivar with pre-attachment *Striga* resistance can be identified and recommended to breeders or farmer communities. The proposed screening method might prove to be a quick and efficient approach that could be highly relevant for future screening and breeding programs and facilitate breeders to screen for pre-attachment resistance

for the development of *Striga* resistant cultivars. Since *Striga* populations are genetically diverse, for durable resistance it would be advisable to combine the pre-attachment resistance reported in this paper with post-germination resistance mechanisms as reported by Cissoko et al. (2011).

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Suppl. Table S1 Amount of strigolactones (orobanchol, 2'-epi-5-deoxystrigol, methoxy-5-deoxystrigol (DS)-isomers 1 to 3; peak area in MRM-LC-MS analysis) in different rice cultivars

Cultivars	orobanchol		2'-epi-5-deoxystrigol		methoxy-5DS- isomer 1		methoxy-5DS- isomer 2		methoxy-5DS- isomer 3	
Name	(10^3)	Rank[a]	(10^3)	Rank	(10^3)	Rank	(10^3)	Rank	(10^3)	Rank
N-1	4.9(13.9)[b]	3	19.3(54.3)[a]	3	1.3(3.5)[a]	10	6.0(16.9)[a]	4	4.1(11.5)[a]	3
N-2	35.5(12.3)	8	174.0(60.4)	21	6.0(2.1)	22	47.2(16.4)	19	25.3(8.8)	10
N-3	16.6(12.2)	4	76.1(56.1)	12	2.6(1.9)	18	19.5(14.4)	10	20.9(15.4)	8
N-4	70.1(50.4)	17	35.5(25.5)	4	0.6(0.4)	6	14.6(10.5)	6	18.3(13.1)	6
N-5	62.9(32.8)	15	67.0(35.0)	10	1.1(0.6)	10	24.6(12.8)	14	36.0(18.8)	13
N-6	73.6(22.1)	18	109.6(32.9)	14	4.9(1.5)	21	69.6(20.9)	22	75.9(22.7)	19
N-7	164.1(40.1)	22	123.7(30.3)	17	3.0(0.7)	19	44.1(10.8)	18	73.9(18.1)	18
N-8	113.4(31.1)	20	116.5(31.9)	15	1.4(0.4)	12	38.7(10.6)	17	95.1(26.0)	21
N-9	66.0(27.8)	16	100.8(42.5)	13	0.4(0.2)	3	21.9(9.2)	11	47.9(20.2)	15
N-10	57.7(18.0)	13	161.3(50.2)	19	2.1(0.7)	16	36.6(11.4)	16	63.8(19.8)	16
N-11	50.6(14.1)	11	163.5(45.4)	20	2.1(0.6)	16	52.3(14.5)	20	91.4(25.4)	20
N-12	19.0(15.3)	5	47.7(38.2)	7	1.9(1.5)	14	23.6(18.9)	12	32.6(26.1)	11
N-13	36.2(23.3)	9	47.6(30.6)	6	1.0(0.6)	9	24.4(15.7)	13	46.3(29.8)	14
N-14	94.6(27.4)	19	147.7(42.8)	18	1.9(0.6)	14	33.3(9.7)	15	67.3(19.5)	17
N-15	125.2(42.8)	21	116.6(39.9)	16	0.6(0.2)	6	16.8(5.8)	8	33.1(11.3)	12
N-16	54.7(45.7)	12	42.7(35.7)	5	0.4(0.3)	3	4.4(3.7)	3	17.5(14.6)	5
N-17	19.1(17.5)	6	56.8(52.2)	9	0.1(0.6)	6	14.9(13.7)	7	17.4(16.0)	4
N-18	43.5(29.2)	10	75.0(50.4)	11	0.4(0.2)	3	10.1(6.8)	5	19.8(13.3)	7
W56-104	4.0(30.3)	2	7.0(53.1)	2	0.1(0.8)	1	0.5(3.5)	2	1.6(12.5)	2
W56-50	60.2(10.7)	14	304.9(54.0)	22	2.7(0.5)	19	57.4(10.2)	21	139.6(24.7)	22
W181-18	30.2(23.1)	7	55.3(42.4)	8	1.4(1.0)	12	19.3(14.8)	9	24.2(18.6)	9
CG14	0.1(6.4)	1	1.0(56.9)	1	0.1(7.4)	1	0.1(3.5)	1	0.5(26.1)	1
P	<0.05		<0.01		<0.05		<0.05		<0.01	
LSD 5%	78.5		128.6		4.4		36.8		54.7	

[1] Ranking was done from 'resistance' to 'susceptible' with rank 1 indicating the highest resistance genotype against *Striga* infection and 22 the most susceptible one.

[a]: Figures in parentheses show the proportion of that strigolactone in the total peak area of strigolactones

Suppl. Table S2 Correlation between various strigolactones

	orobanchol	2'-epi-5-deoxystrigol	methoxy-5DS-isomer 1	methoxy-5DS-isomer 2	methoxy-5DS-isomer 3
orobanchol					
2'-epi-5-deoxystrigol	0.43 *				
methoxy-5-deoxystrigol-isomer 1	0.17 ns	0.55 **			
methoxy-5-deoxystrigol-isomer 2	0.45 *	0.78 ***	0.79 ***		
methoxy-5-deoxystrigol-isomer 3	0.54 **	0.84 ***	0.39 ns	0.83 ***	

*P <0.05; **P <0.01; ***P <0.001; ns: non-significant

Chapter 4

Genetic variation in strigolactone production and tillering in rice and its effect on *Striga hermonthica* infection

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Abstract

Tillering in cereals is a complex process in the regulation of which also signals from the roots in the form of strigolactones play an important role. The strigolactones are signalling molecules that are secreted into the rhizosphere where they act as germination stimulants for root parasitic plants and hyphal branching factors for arbuscular mycorrhizal fungi. On the other hand, they are also transported from the roots to the shoot where they inhibit tillering or branching. In the present study the genetic variation in twenty rice varieties collected from all over the world in strigolactone production and tillering phenotype was studied and related with *Striga* infection. Rice cultivars like IAC 165, IAC 1246, Gangweondo and Kinko produced high amounts of the strigolactones orobanchol, 2'-epi-5-deoxystrigol and three methoxy-5-deoxystrigol isomers and displayed low amounts of tillers. These varieties induced high *Striga* germination, attachment, emergence as well as dry biomass. In contrast to this, rice cultivars such as Super Basmati, TN 1, Anakila and Agee displayed high tillering in combination with low production of the above mentioned strigolactones. These varieties induced only low *Striga* germination, attachment, emergence and dry biomass. Statistical analysis across all the varieties confirmed a positive correlation between strigolactone production and *Striga* infection and a negative relationship with tillering. These results show that genetic variation in tillering capacity is the result of genetic variation in strigolactone production and hence could be a helpful tool in selecting rice cultivars that are less susceptible to *Striga* infection.

Keywords: Rice, strigolactones, *Striga,* tillering

Introduction

A tiller is a specialized grain bearing stem that sprouts from the base of plant species in the *Poaceae*. Tillering is one of the most important agronomic traits in poaceous crops and plays a major role in determining plant architecture and grain yield (Wu et al. 1998). Tillering (and branching in dicotelydonous crops) is a complex process that involves the fine-tuned, coordinated expression of many genes (Ongaro and Leyser 2007) and is regulated by the interaction of genetic, hormonal and environmental cues (Ward and Leyser 2004; Garba et al. 2007; Kim et al. 2010a, b). Initially, two classes of hormones - auxins and cytokinins – have long been assumed to control tillering and shoot branching (Leyser 2003; Hayward et al. 2009). But recently a novel class of plant hormones, the strigolactones, was identified to also be involved in the regulation of above-ground plant architecture by inhibiting tiller production/shoot branching (Umehara et al. 2008; Gomez-Roldan et al. 2008; Crawford et al. 2010; Umehara et al. 2010). The production of strigolactones is particularly strongly up-regulated under low phosphate conditions (Yoneyama et al. 2007b; Jamil et al. 2011a). Under phosphorus deficiency, the modification in plant architecture by strigolactones (reduced tillering/shoot branching) is proposed to be an adoptive strategy to these growth limiting conditions (Umehara et al. 2010; Kohlen et al. 2011). In addition, there is increasing evidence that under phosphorus limitation, strigolactones also play a role in the adaptation of root architecture to better cope with this nutrient limitation (Koltai et al. 2010; Kapulnik et al. 2011a; Ruyter-Spira et al. 2011). Another adaptation to improve uptake of mineral nutrients in which strigolactones play a key role – but now not as plant hormone but as rhizosphere signalling molecule - is the stimulation of the symbiotic interaction with arbuscular mycorrhizal (AM) fungi (Akiyama et al. 2005). The strigolactones act as a hyphal branching factor for AM fungi and hence stimulate the development of a symbiotic relation (Akiyama et al. 2005). The AM fungi, play an important role in providing mineral nutrients especially phosphorus to more than 80% of land plant species (Harrison 2005; Akiyama and Hayashi 2006; Bouwmeester et al. 2007).

However, the strigolactones have a second rhizosphere signalling role. They also act as germination stimulants for seeds of root parasitic plants of the *Striga*, *Orobanche* and *Phelipanche* genera, resulting in a parasitic interaction between host and parasite (Bouwmeester et al. 2003; Xie et al. 2010). The seeds of these parasitic plants will only germinate after perceiving the germination stimulant from their host (Bouwmeester et al. 2003; Yoneyama et al. 2010). After germination, the parasite attaches and penetrates the host root by a specialized feeding structure, the haustorium (Lynn and Chang 1990; Estabrook and Yoder 1998; Yoder 2001). The parasitic plant grows underground for 4 to 7 weeks prior to emergence and utilizes host water, nutrients and photosynthates. Much of the damage to the host occurs already at this stage. About 20-80% yield losses or even complete crop failure can occur due to this parasitism.

The triple role of strigolactones in underground communication between host plants, AM fungi and parasitic plants, and the regulation of tillering/branching raises a number of questions. One of these is if the tillering/branching phenotype is indicative of the production of strigolactones and their secretion into the rhizosphere - and whether then the tillering phenotype is also an indication of the plant's susceptibility to root parasitic plant infection. If this is the case, screening of cultivars for their tillering/branching phenotype could be an easy tool for breeders to select lines with better parasitic plant resistance. In the present study we applied this question to rice with the aims to correlate rice tillering with strigolactone production and to link this feature with *Striga* infection in a range of rice cultivars from all over the world.

Material and methods

Plant material

In a first study, about 50 rice cultivars collected from different areas of the world were studied for strigolactone production and tillering capacity. Out of these 50 rice cultivars, 20 rice cultivars were selected for further experiments based on the variation in strigolactone production, tillering and *Striga* germination, attachment and emergence. Details of selected twenty rice cultivars are given in Table 1.

Table 1 List of rice cultivars screened for tillering, strigolactones production and *Striga* infection

S. No.	Variety name	Rice species	Group	Seed source	Country of origin	Tall/Dwarf	Tillering (No. plant^{-1})	Upland/ lowland
1	IAC 165	*O. sativa*	Japonica	Uni. Shef.	Brazil	Tall	6	upland
2	IAC 1246	*O. sativa*	Japonica	IRRI	Brazil	Tall	6	upland
3	Gangweondo	*O. sativa*	Japonica	IRRI	South Korea	Tall	7	No info.
4	Kinko	*O. sativa*	Indica	IRRI	Guinea	Tall	7	No info.
5	Dullo	*O. sativa*	Indica	IRRI	India	Tall	8	No info.
6	Binagimbing	*O. sativa*	Indica	IRRI	Philippines	Intermediate	8	upland
7	Sonkanoir	*O. sativa*	Indica	IRRI	Liberia	Intermediate	8	No info.
8	20 D	*O. sativa*	Indica	IRRI	Liberia	Intermediate	9	upland
9	TOS 7556	*O. sativa*	Javanica (Tropical japonica)	IRRI	Ivory Coast	Intermediate	8	upland
10	Kairyo-HM	*O. sativa*	Japonica	IRRI	Japan	Intermediate	9	No info.
11	PI 160641	*O. sativa*	Indica	IRRI	China	Intermediate	11	No info.
12	Koirao Baleo	*O. glaberrima*	-[a]	IRRI	Mali	Intermediate	11	upland
13	Bhasmanik	*O. sativa*	Indica	IRRI	India	Intermediate	12	No info.
14	Shuang-Chiang	*O. sativa*	Indica	IRRI	Taiwan	Intermediate	12	No info.
15	Peh-Kuh	*O. sativa*	Indica	IRRI	Taiwan	Dwarf	13	No info.
16	Tattare	*O. glaberrima*	-	IRRI	Nigeria	Dwarf	12	No info.
17	Agee	*O. glaberrima*	-	IRRI	Ghana	Dwarf	12	No info.
18	Anakila	*O. glaberrima*	-	IRRI	Mali	Dwarf	14	No info.
19	TN 1	*O. sativa*	Indica	IRRI	Taiwan	Dwarf	16	Intermediate
20	S. Basmati	*O. sativa*	Indica	NARC	Pakistan	Dwarf	18	Lowland

[a] Japonica/Indica/Javanica are different eco-geographic groupings of *Oryza sativa* only, not applied to *Oryza glaberrima*

The rice cultivars represent different origins of the world especially Africa and Asia and represent *Oryza japonica*, *Oryza indica* and *Oryza javanica* backgrounds. All the experiments were conducted

in a completely randomized design with three replicates under controlled greenhouse conditions (28°C/25°C with 10h (day)/14h (night) photoperiod and 70% relative humidity) or in a climate chamber (28°C/25°C with 10h (day)/14h (night) photoperiod (450 µM m^{-2} s^{-1}) and 70% relative humidity) in Wageningen, the Netherlands.

Strigolactone analysis

Strigolactones were analyzed in root exudates as well as in root extracts. The seeds of all cultivars were surface sterilized with 2% sodium hypochlorite and placed to germinate in an incubator at 30°C for 48 h. About 1.5 L silver sand was added to a 3.0 L plastic pot and 15 pre-germinated seeds of each cultivar, in 3 replicates, were planted in an individual pot and grown in the climate chamber. During the 2nd week, thinning was done to 10 plants in each pot. Each pot was supplied with 250 mL half strength modified Hoagland's nutrient solution with 100% phosphorus (P; 0.4 mM) for four weeks (at 48 h interval). In week 5, 3 L phosphorus deficient nutrient solution was added on top of the sand and allowed to drain from each pot. The phosphorus deficiency was maintained for one week, after which another 3 L phosphorus deficient nutrient solution was applied and drained away freely to remove accumulated strigolactones from the rhizosphere. After 48 h, root exudates were collected from the rhizosphere of each pot by adding and draining 1.5 L phosphorus deficient nutrient solution through each pot. The collected solutions were passed through SPE C18-Fast columns (500 mg/3 mL) and the strigolactones eluted with 6 mL 100% acetone. Various strigolactones such as orobanchol, 2'-epi-5-deoxystrigol and three methoxy-5-deoxystrigol isomers (Fig. 1) were identified and quantified using ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) (Waters, Milford MA USA) as described previously (Jamil et al. 2010).

Strigolactones were also measured in root extracts of the first four highest strigolactone producing and last four lowest producing rice cultivars. Hereto, root tissues from these cultivars were frozen with liquid nitrogen and ground into powder. About 500 mg of ground powder of each sample was taken in a pre-cooled glass vial. Then 4 mL ethyl acetate and 0.02 nmol of D_6-2'-epi-5-deoxystrigol (provided by T. Asami, Department of Applied Biological Chemistry, The University of Tokyo, Japan) as internal standard were added and the samples sonicated for 10 min. After sonification and vortexing, the samples were centrifuged at 2000 *g* for ten min. The supernatant was collected carefully and added to a new glass vial. The strigolactones collected in this way, were identified and quantified through Ultra Performance Liquid Chromatography coupled to tandem Mass Spectrometry (UPLC-MS/MS) using Multiple Reaction Monitoring (MRM) as described by (Jamil et al. 2011a). The retention times and mass transitions of strigolactone standards orobanchol and 2'-epi-5-deoxystrigol (provided by Koichi Yoneyama, Weed Science Center, Utsunomiya University, Japan) and 5-deoxystrigol (provided by Kohki Akiyama, Osaka Prefecture University, Japan) were used to identify rice strigolactones. Strigolactones putatively identified as methoxy-5-deoxystrigol isomers 1-

3 (Jamil et al. 2011a; Cardoso, personal communication) were also identified by using MRM transitions (channels) *m/z* 361>247 and 361>97.

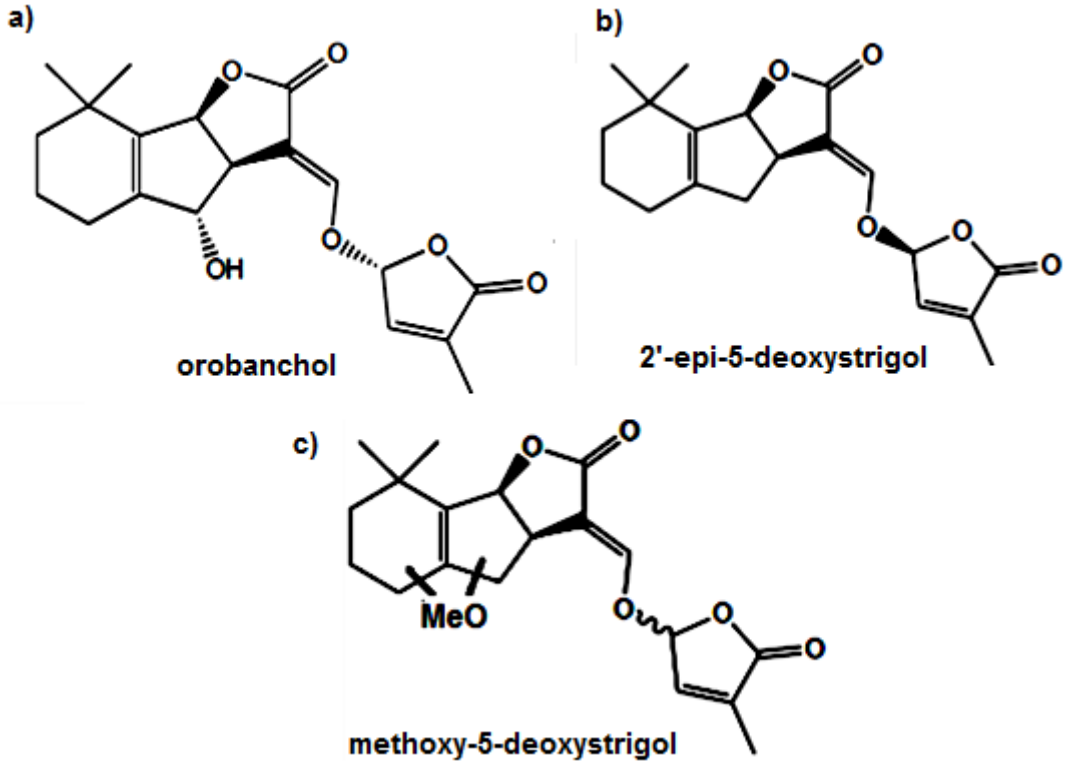

Fig. 1 Chemical structure of orobanchol (**a**), 2'-epi-5-deoxystrigol (**b**) and methoxy-5-deoxystrigol isomers 1 to 3 (position and stereochemistry unknown) (**c**) in the root exudates and root extracts of rice cultivars (Adapted from Jamil et al. 2011a)

Tillering diversity among rice cultivars

To determine the tillering diversity, the seeds of each rice cultivar were surface sterilized with 0.2% sodium hypochlorite and put to germinate in separate sealed Petri dish in an incubator at 30ºC for 48 h. One pre-germinated seed of each rice cultivar was planted in the center of a 1 L plastic pot, filled with 800 mL silver sand. The rice seedlings in this completely randomized design experiment with three replicates were allowed to grow under green house controlled conditions as mentioned above. Half strength Hoagland's nutrients solution was applied (250 mL at 48 h interval) with 100% phosphorus (0.4 mM) and then after one week the dose of P reduced to 20% (0.08 mM) to induce strigolactone production. To determine number of tillers per plant, the plants were grown for about eight weeks (58 days). The tillers per plant of each cultivar were counted manually from each pot.

***Striga* germination bioassays**

Root exudates obtained from different rice cultivars were assessed for germination stimulatory activity by germination bioassays with seeds of *Striga*. Surface-sterilization of *Striga* seeds was done using 25 mL of 2% sodium hypochlorite with 0.4% of Tween-20 for 5 min. Subsequently, seeds were thoroughly rinsed three times with 10 min intervals, using sterile demineralized water, through a Buchner funnel. The sterile seeds were air dried for sixty min. Approximately 50 to 100 seeds were then evenly spread on 9-mm diameter glass fiber filter paper discs (Sartorius, Goettingen Germany). These discs were placed in 9-cm diameter Petri-dishes (12 discs per Petri-dish) on filter paper (Whatman, Maidstone England) moistened with 3 mL demineralized water. For preconditioning, the Petri-dishes were sealed with parafilm, wrapped in aluminium foil and placed in an incubator in darkness at 30ºC for 10 days.

After 10 days, the discs with preconditioned seeds were allowed to dry for 50 min in a laminar flow cabinet to evaporate surplus moisture. The discs were then placed in another Petri-dish (six per Petri-dish) containing a filter paper ring (outer diameter 9 cm, inner diameter 8 cm) moistened with 0.9 mL water. The samples to be tested were applied (50 µL per disc) in 3 replicates after replacement of the acetone in the samples by water through vacuum centrifugation. GR24 (3.3 µM) was used as a positive control and water as a negative control in each germination assays. Seeds were again incubated at 30ºC in darkness for 48 h and germination (seeds with radicle protruding through the seed coat) was scored using a binocular (Matusova et al. 2005).

***Striga* attachment**

The response of high and low tillering cultivars for *Striga* attachment was assessed in a pot study. All rice cultivars were grown in a completely randomized design with three replicates. A perforated plastic sheet was put in the bottom of 1.5 L plastic pot. After addition of 100 mL *Striga* free silver sand on the bottom of the pot, a mixture of 500 mL sand with 25 mg of *Striga* seeds was placed on top of the *Striga*-free layer. One pre-germinated seed of each rice cultivar was planted in the center of each pot and covered by another 100 mL of *Striga* free sand. During the first week, half strength modified Hoagland's nutrient solution with 100% P was applied (250 mL at 48 h interval). Subsequently, the phosphorus dose was reduced to 20% and 250 mL of this nutrient solution was applied at 48 h intervals. The plants were grown for eight weeks under greenhouse conditions as mentioned above. After 8 weeks, sand was removed carefully by washing the root systems of the rice plants and *Striga* attachment counted under a binocular.

Striga emergence

Striga emergence and dry biomass production were assessed in a separate experiment. A mixture of soil (Lentse Potgrond, Katwijk the Netherland) and silver sand in a ratio of 1:1 was prepared. About 200 mL of this medium without *Striga* seed was added in the bottom of a 1.5 L plastic pot and then 500 mL of this medium, mixed with 25 mg *Striga* seeds was added. Finally 100 mL of above mentioned medium without *Striga* seed was added on top. A pre-germinated seed of each cultivar was planted in the center of each pot. The plants were grown under the greenhouse conditions described above for 12 weeks. After counting *Striga* emergence the *Striga* plants were up-rooted, oven dried at 70^{o}C for 72 h and then weighed.

Statistical analysis

The data on *Striga* germination, attachment, emergence and dry biomass were analyzed statistically by ANOVA in conjunction with LSD Posthoc test using Genstat 9.2 (VSN international Ltd. Hemel Hempstead England). The relationship between various strigolactones and *Striga* germination and tillering was analysed by correlation analysis using R. For a multivariate approach, the average strigolactone peak areas of the rice cultivars were symmetrically scaled by the standard deviation (SD) of the samples and the individual strigolactones. The STD-scaled data were used (without log transformation) in redundancy analysis (RDA), to examine whether the variation in the strigolactones explains the variation in average infection parameters of the rice genotypes. We used a forward selection approach to identify the strigolactones with the highest explanatory power. A Monte Carlo Permutation (MCP) test was used to assess the statistical significance of the ordination axes of the canonical analysis and of the environmental variables. All the multivariate data analysis was performed in CANOCO 4.5 for windows (ter Braak 1988).

Results

Tillering

The rice cultivars differed strongly with regard to their tillering phenotype (Fig. 2). The average number of tillers varied from 6 to 18 tillers per plant (Fig. 3; Suppl. Table S1). The rice cultivars Super Basmati, TN 1, Agee, and Anakila showed the highest tillering (13-18 tillers per plant) while rice cultivars such as IAC 165, IAC 1246, Gangweondo and Kinko displayed much lower number of tillers per plant (Fig. 2; Fig. 3). The rest of the cultivars were in the range of 8-12 tillers per plant (Suppl. Table S1).

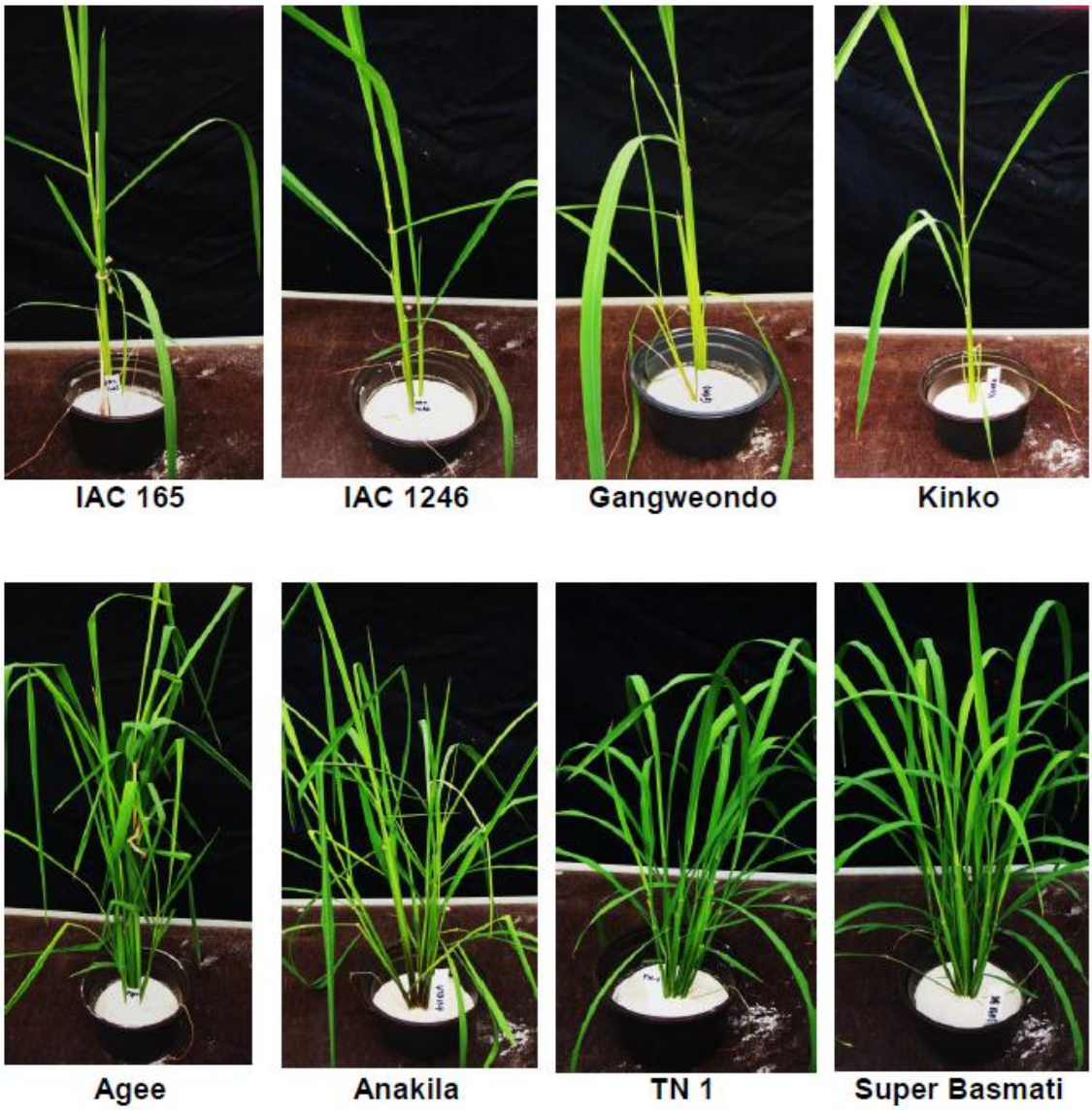

Fig. 2 Differences in tillering in some representative low and high tillering rice cultivars. The average number of tillers (between brackets) at eight weeks after sowing were: IAC 165 (6), IAC 1246 (6), Gangweondo (7), Kinko (7), Agee (12), Anakila (14), TN 1 (16) and Super Basmati (18)

Striga infection

The exudates of low tillering rice cultivars IAC 165, IAC 1246, Gangweondo, and Kinko induced the highest *Striga* germination rate (≥50%), while high tillering rice cultivars like Super Basmati, TN 1, Anakila and Agee induced significantly less germination of *Striga*. Bhasmanik, Shuang, Pehkuh, Tattare were the other four cultivars inducing less than 25% germination (Fig. 3; Suppl. Table S1). The four lowest tillering rice cultivars with high *in vitro* germination rates (IAC 165, IAC 1246, Gangweondo and Kinko) also had the highest *Striga* attachment. In contrast, the high tillering rice cultivars, with the lowest germination rates (Super Basmati, TN 1, Anakila and Agee), had the lowest number of *Striga* attachments (Fig. 3). For most of the other cultivars, *Striga* attachment was similar to their position based on germination rate (Suppl. Table S1).

In line with the germination and attachments results, the four low tillering rice cultivars also displayed the highest *Striga* emergence (>25 plants per pot) while high tillering cultivars as Agee, Anakila, TN 1 and Super Basmati showed significantly (P<0.01) lower *Striga* emergence numbers (<4 plants per pot) (Fig. 3; Fig. 4).

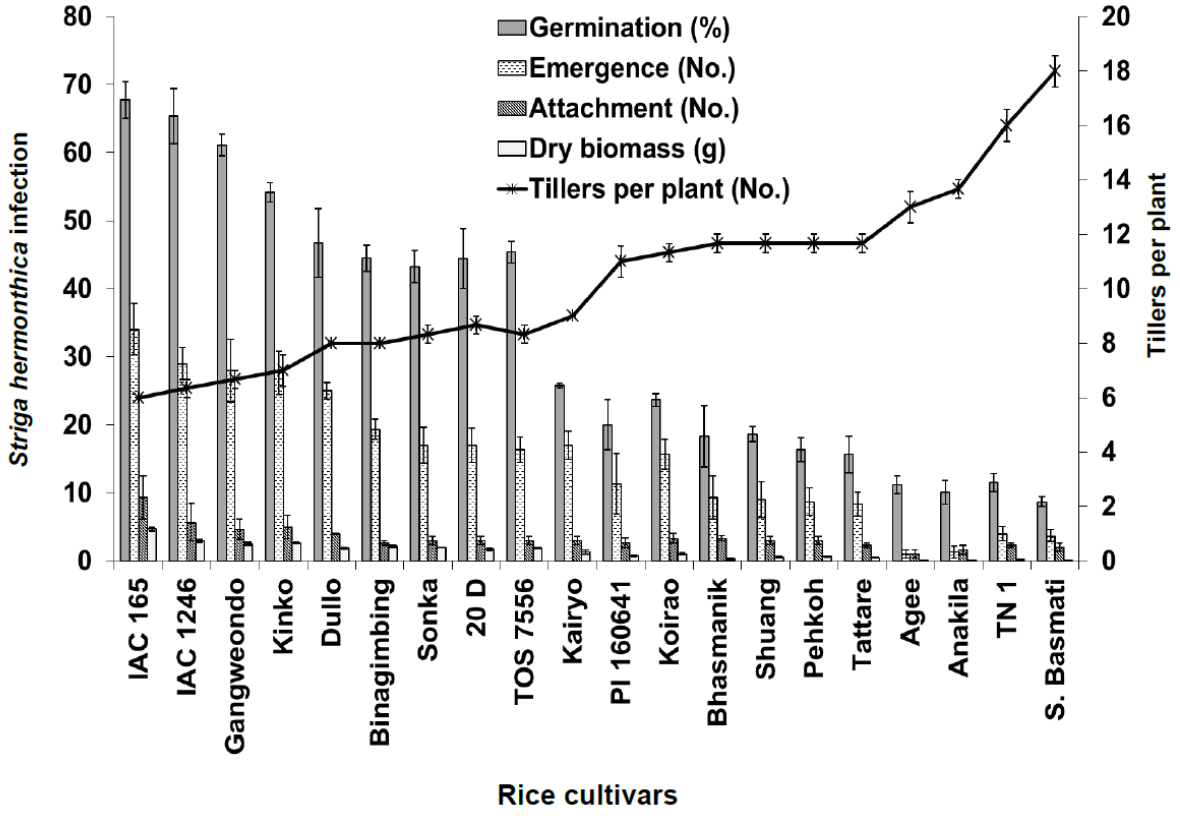

Fig. 3 Numbers of tillers, *Striga* germination (%) induced by root exudate, attachment (No.), emergence (No.) and dry biomass (g) of *Striga* in a series of rice cultivars. Bars represent means±SE (n=3). The line represents average number of tillers per plant±SE (n=3)

The rest of the rice cultivars showed intermediate *Striga* emergence (8-25 plants per pot) (Suppl. Table S1). In accordance with the high *Striga* emergence, the low tillering rice cultivars, IAC 165, IAC 1246, Gangweondo and Kinko supported the highest *Striga* biomass (Fig. 3), while the lowest *Striga* biomass was found on the high tillering rice cultivars Super Basmati, TN 1, Anakila and Agee. The latter varieties supported significantly (P<0.01) lower *Striga* biomass than 80% of the other cultivars. The other moderate tillering rice cultivars, like Tattare, Pehkuh, Shuang, Bhasmanik, Koirao and PI 160641 showed significantly lower *Striga* dry biomass (<600 mg) than 50% of the other cultivars (Suppl. Table S1).

Strigolactone production

The concentration of strigolactones in the exudate varied strongly between the rice cultivars (Fig. 5a; Suppl. Table S2). The low tillering cultivars IAC 165, IAC 1246, Gangweondo, and Kinko were found to be top four highest strigolactone producers (Fig. 5a). The cultivars Dullo, Binagimbing,

Sonka, 20D, TOS 7556, Kairyo, Tattare, Shuang, Pehkoh and Bhashmanik showed intermediate production levels while the high tillering cultivars Super Basmati, TN 1, Anakila and Agee were found as lowest strigolactone producers (Fig. 5a; Suppl. Table S2). Also the endogenous concentration of strigolactones in roots was high in the low tillering cultivars IAC 165, IAC 1246, Gangweondo and Kinko and low in the high tillering cultivars Super Basmati, TN 1,

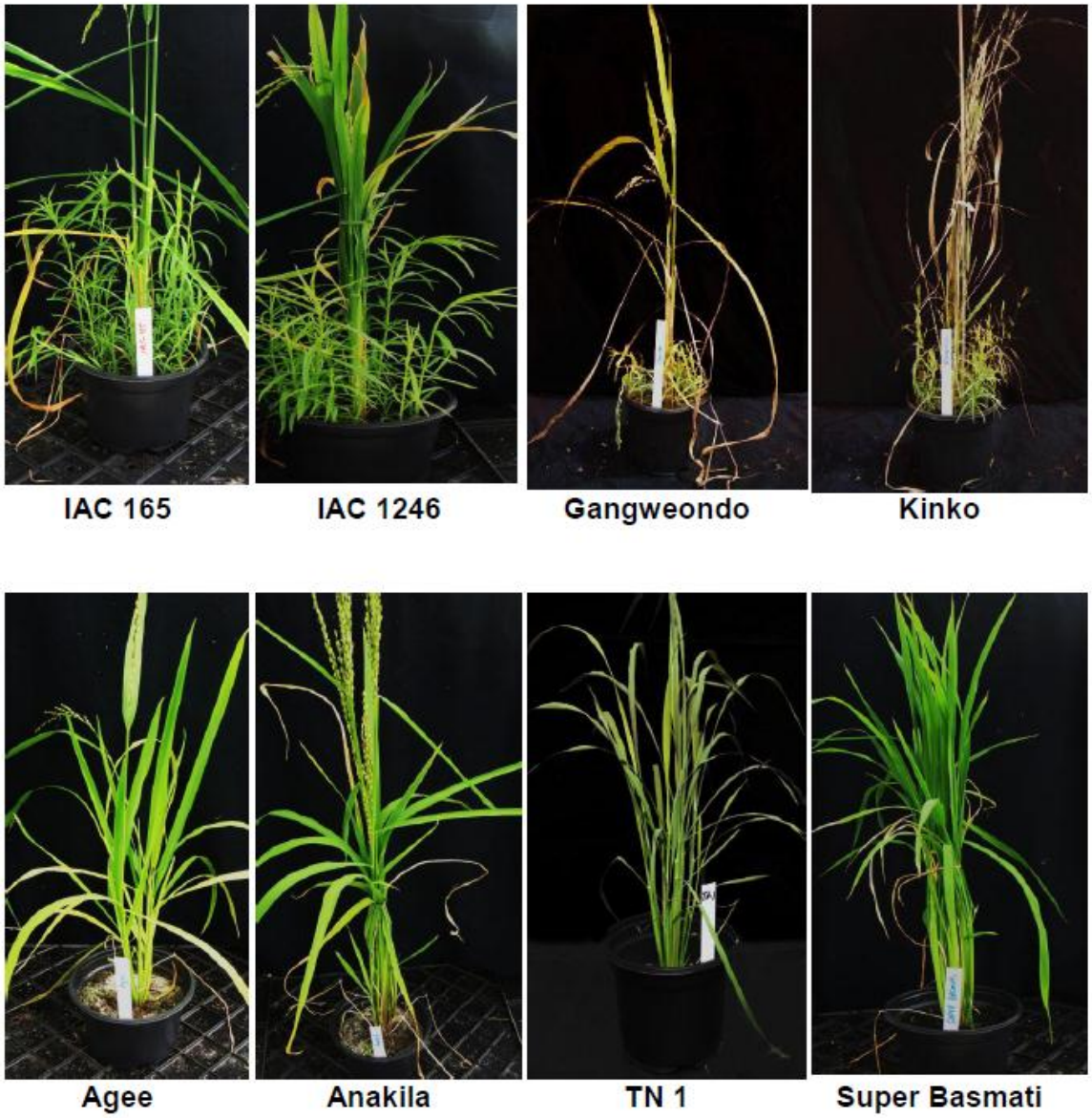

Fig. 4 *Striga* emergence in some representative low and high tillering rice cultivars as determined 12 weeks after sowing. The average number of *Striga* shoots emerged (between brackets) were: IAC 165 (34), IAC 1246 (29), Gangweondo (28), Kinko (28), Agee (1), Anakila (1), TN 1 (4) and Super Basmati (4)

Anakila and Agee (Fig. 5b). In addition to differences in the total amount of strigolactones, the cultivars also showed differences in the composition of strigolactones (Figs 5a, b). The four cultivars producing the highest amount of 2'-epi-5-deoxystrigol included Gangweondo, IAC 165, Kinko and IAC 1246 (Figs 5a, b; Suppl. Tables S2 and S3). For orobanchol, the highest producing cultivars were IAC 165, IAC 1246, Dullo and Kinko (Fig. 5a). The cultivars IAC 165, IAC 1246, Gangweondo

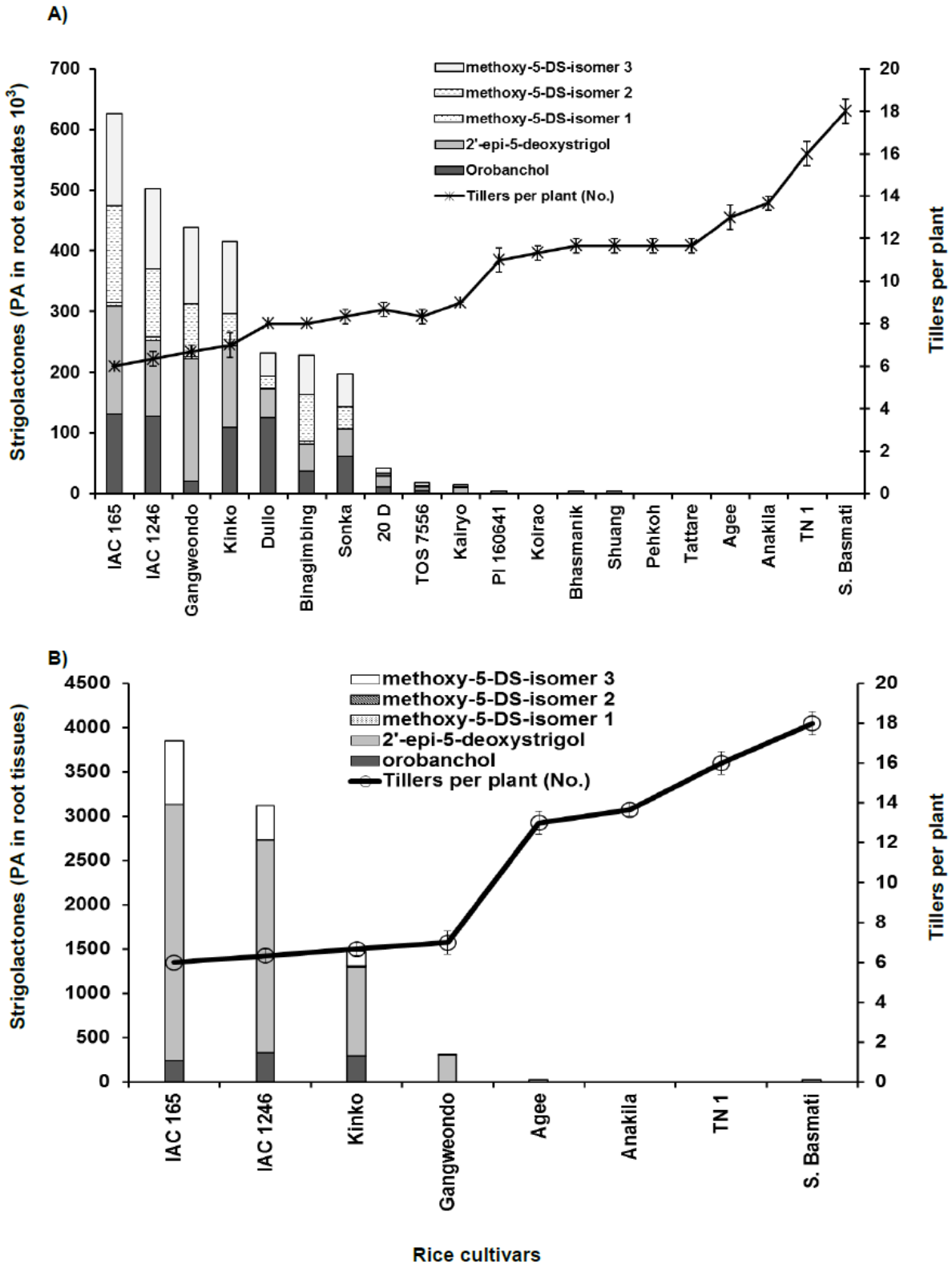

Fig. 5 Strigolactone concentration in the root exudates (a) and root tissues (b) of rice cultivars: 2'-epi-5-deoxystrigol, orobanchol and methoxy-5-deoxystrigol isomers 1-3. Bars represent means of peak areas of the individual strigolactones as determined by Multiple Reaction Monitoring Liquid Chromatography-Mass Spectrometry in triplicate. The line represents the average number of tillers per plant±SE (*n*=3)

and Kinko produced high levels of at least two of the three methoxy-5-deoxystrigol isomers 1-3 (Fig. 5a, b). The rice cultivar IAC 165 ranked on top for the amount of orobanchol and methoxy-5-deoxystrigol isomers 1-3 in the root exudate but in the root itself contained the highest amount of 2'-epi-5-deoxystrigol (Figs 5a, b).

Relationship between tillering, strigolactone production and *Striga* infection

The above data show that there is an inverse relationship between the number of tillers per plant and *Striga* parasitism parameters (germination, attachment, emergence, dry biomass) as well as strigolactone production (Fig. 3; Fig. 5a, b). Regression analysis (on the basis of peak area) revealed that methoxy-5-deoxystrigol isomer 3 contributes most to the explanation of the variation in *Striga* germination and emergence in these rice cultivars followed by 2'-epi-5-deoxstrigol (Suppl. Table S4). The strigolactones did not contribute significantly to the explanation of variation in *Striga* attachment (Suppl. Table S4). 2'-Epi-5-deoxystrigol also contributes significantly to the explanation of variation in tiller numbers in contrast to the other strigolactones. However, correlation analysis showed that 2'-epi-5-deoxystrigol, orobanchol and the methoxy-5-deoxystrigol isomers 1-3 also closely correlate with each other (and *Striga* germination, attachment and emergence) (Suppl. Table S6). Interestingly, there was a higher correlation between 2'-epi-5-deoxystrigol and the three methoxy-isomers (0.77-0.81) than between orobanchol and these three isomers (0.60-0.66). The correlation between orobanchol and 2'-epi-5-deoxystrigol was in the same range as between orobanchol and the three isomers (0.63).

Statistical analysis of the variation between rice cultivars

The correlations between explanatory variables (strigolactones) and response variables (infection parameters) were visualized in an RDA triplot. The high strigolactone producing rice cultivars IAC 165, IAC 1246, Gangweondo, Kinko and Dullo clustered together along the first ordinate on the right side of the plot whereas low producers such as Kairyo, PI 160641, Koirao, Bhasmanik, Peh-Kuh, Shuang, Tattare, Agee, Anakila, TN 1 and Super Basmati clustered tightly together along the first ordinate on the left side of the plot. The remaining rice cultivars Binagimbing, Sonkanoir and 20 D located more or less in between these two clusters forming a group of moderate strigolactone producers.

The strigolactones explained 79% of the variance in infection parameters and tillering (MCP, *P*-value < 0.01) which is close to the explained variance by the first ordinate (78.8%). There is a high correlation between the individual strigolactones as illustrated by the fact that their vectors point in almost the same direction along the first ordinate. Only the vector of orobanchol deviates slightly from the others. Due to this high correlation, each strigolactone explained a significant and sizeable fraction of the variance in infection parameters and tillering when only one strigolactone was added to

the ordination model, with methoxy-5-deoxystrigol-isomer-3 (Meth-3) explaining the highest (76%, MCP, *P*-value < 0.01) and orobanchol explaining the lowest fraction of the variation (61%, MCP, *P*-value < 0.01). However forward selection showed that after addition of the most explanatory strigolactone (Meth-3) to the ordination model, orobanchol is the most explanatory strigolactone for the rest of the variation (2% MCP, *P*-value = 0.2) (Suppl. Table S6). All the strigolactones correlated negatively with tillering and positively with *Striga* germination. The strigolactones also correlated positively, with attachment and emergence but less strong than with germination (Fig. 6) suggesting that there are other factors than the strigolactones influencing attachment and emergence of *Striga* after germination as for example in rice cultivar Dullo. Despite the similar total level of strigolactones exuded (and the similar germination), Dullo supported higher *Striga* emergence and attachment than Binagimbing (Fig. 6; Suppl. Tables S1-S2). Both have quite similar total amount of strigolactones but quite different composition (Fig. 5). Also among the other varieties there is strong variation in the strigolactone composition.

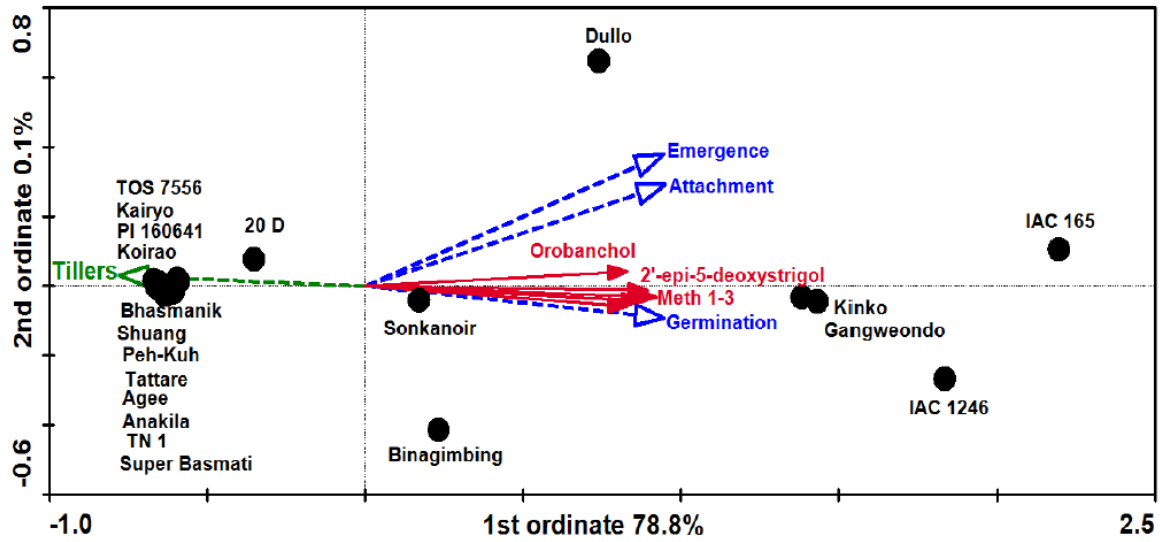

Fig. 6 Redundancy analysis plot visualizing the distance between rice cultivars (solid circles) based on a direct gradient analysis and showing patterns in the parasitism and tillering parameters (dotted arrows, response variables) that could be explained by the strigolactones (solid arrows, explanatory variables). The ordination axes are aggregates of the explanatory variables that best explain the response data

This is visualized by the ratio's between the individual strigolactones (Suppl. Fig. 1; Suppl. Table 5). For example, PI160641 produces much more orobanchol than 2'-epi-5-deoxystrigol whereas for Gangweondo this is the reverse (Suppl. Fig. 1a), suggesting differential activity of the two branches of the strigolactone pathway in these cultivars. The low tillering rice cultivars IAC 165 and Gangweondo have much lower orobanchol to 2'-epi-5-deoxystrigol ratio both in the root exudate and in the root tissue compared with the high tillering Agee and Super Basmati (Suppl. Table S5). Intriguingly, there is also variation for the ratio between strigolactone concentration in the exudate

and in the root (Figs 5 and 6; Suppl. Table S5). Agee, for example, has a high orobanchol/2'-epi-5-deoxystrigol ratio in the root, whereas in the exudate it is average. For TN1 it is more or less the opposite. Another interesting feature in the graphs is that there seems to be no consistent relation between orobanchol and the methoxy-isomers (Suppl. Fig. 1 b-c), and that in Bhasmanik the (putative) conversion of 2'-epi-5-deoxystrigol to both methoxy-isomers is highly active (Suppl. Fig. 1 d-e).

Discussion

The LC/MS quantification of strigolactones and the assessment of tillering and *Striga* infection in a range of rice cultivars collected from all over the world in the present study confirms the correlation between the regulation by strigolactones of above ground plant architecture (tillering) and *Striga* infection that has been demonstrated before (Gomez-Roldan et al. 2008; Umehara et al. 2008, 2010). We show that there is extensive genetic variation for strigolactone production among the twenty rice varieties investigated and that this genetic variation is reflected in strong variation in tillering and *Striga* infection. Low tillering, high strigolactone producing rice cultivars are more prone to *Striga* infection, and high tillering, low strigolactone producing varieties are less prone to *Striga* infection. This shows that genetic variation for strigolactone production is available in rice, opening up possibilities to introduce the low *Striga* germination induction trait in rice. The results of the present study fit well with the postulated hypothesis that low secretion of strigolactones into the rhizosphere could be used to reduce *Striga* infection (Bouwmeester et al. 2003; Sun et al. 2008). Our results with rice match with results on sorghum, showing that cultivars that produce low levels of the germination stimulants are resistant to *Striga* (Ejeta 2007; Mohamed et al. 2010). For rice, our study could imply that the introduction of more-tillering cultivars (with low strigolactone production) may prove to be an inexpensive approach to *Striga* management in rice, an increasing problem in parts of the African continent (Rodenburg et al. 2006a; Rodenburg and Johnson 2009; Rodenburg et al. 2010).

In addition to genetic variation in strigolactone amount, we also found variation in the strigolactone composition. The strigolactones that we analyzed – orobanchol, 2'-epi-5-deoxystrigol and the methoxy-5-deoxystrigol isomers 1 to 3 (Fig. 1) can be divided into two groups based on their correlation to each other (Suppl. Table S5): orobanchol groups separately from the other four that exhibit a much higher correlation to each other. This is also visualized in Fig. 6 where the vector for orobanchol deviates from those of the other four strigolactones. The exact identity of the methoxy-5-deoxystrigol isomers has not yet been determined, but the higher correlation with 2'-epi-5-deoxystrigol could suggest that they have a 2'-epi-coupled D-ring which would make them methoxy-2'-epi-5-deoxystrigol isomers. Purification of high enough amounts of these isomers followed by NMR characterization should prove this assumption. The biosynthesis of orobanchol and the

methoxy-2'-epi-5-deoxystrigol isomers would diverge from the step in which the D-ring is coupled/formed to produce 5-deoxystrigol - the precursor for orobanchol - and 2'-epi-5-deoxystrigol, the precursor for the methoxy-2'-epi-5-deoxystrigol isomers. Interestingly, we cannot detect 5-deoxystrigol (or only in minute amounts) suggesting that the conversion to orobanchol is very efficient and not rate-limiting. Nevertheless, there is substantial genetic variation in the rice varieties included in the present study in the ratio between orobanchol and 2'-epi-5-deoxystrigol and the methoxy-5-deoxystrigol isomers (Suppl. Table S5; Suppl. Fig. 1). This large variation suggests that there is genetic variation in the stereochemistry of the D-ring coupling/formation. Similarly, the absence of a pattern in the orobanchol/methoxy-5-deoxystrigol isomer ratio's is again suggestive that they might be methoxy-2'-epi-5-deoxystrigol isomers (Suppl. Table S5; Suppl. Fig. 1). Intriguing is the variation for the ratio between the strigolactone concentrations in the exudate and in the root (Figs 5a, b; Suppl. Table S5). This difference in the ratio in root exudates and root tissues is suggestive of selectivity in the transport of strigolactones to the rhizosphere (to attract AM fungi) or to the shoot (for tillering inhibition). It has been demonstrated that different strigolactones exhibit different activities in the biological processes they control (branching inhibition, AM fungi hyphal branching and germination of parasitic plant seeds (Kohlen et al. 2011c) so differentiation in transport to the shoot or the rhizosphere could be biologically meaningful. Indeed, the presence of orobanchol in the xylem of *Arabidopsis thaliana* and tomato has recently been reported whereas other strigolactones present in the root exudate were not detectable in the xylem (Kohlen et al. 2011a).

In the RDA analysis, strigolactones explained a considerable fraction of the variation in infection parameters and tillering (79%). In the RDA plot, the strigolactones correlate strongly positively with germination, attachment and emergence of *Striga* and negatively with tillering of the rice cultivars. Germination significantly correlated positively with 2'-epi-5-deoxystrigol, orobanchol and methoxy-5-deoxystrigol isomer-3 (Suppl. Table S6). In *in vitro* germination bioassays with standards of orobanchol and 2'-epi-5-deoxystrigol, germination of *Striga* was induced more efficiently by 2'-epi-5-deoxystrigol than by orobanchol (data not shown). Germination bioassays with fractions of rice root exudate suggest that the methoxy-5-deoxystrigol isomers are also highly effective in inducing germination of *Striga* (Cardoso, WUR, personal communication). The regression analysis in the present study further confirms this tendency. Methoxy-5-deoxystrigol isomer 3 contributed most to the explanation of the variation in *Striga* germination followed by 2'-epi-5-deoxystrigol while orobanchol did not contribute (Suppl. Table S4). The correlation of attachment and emergence with the amount of strigolactones is lower than for germination but still considerable. Clearly, not all germinated *Striga* seeds will also emerge. The induction of *Striga* seed germination by the strigolactones is just the first step in the parasitation process. Subsequently, successful attachment and a compatible interaction are requirements for emergence. The difference in the direction of emergence and germination arrows with attachment points to differences in resistance between the rice varieties

in these later stages of the parasitation process (Fig. 6). Differences in resistance in the germination phase as well as in post-attachment resistance were recently also reported for the New Rice for Africa NERICA's (Cissoko et al. 2011; Jamil et al. 2011c).

AM fungal symbiosis in high tillering rice cultivars might be affected negatively by the lower production of strigolactones which could cause reduced hyphal branching in the root zone (Garcia-Garrido et al. 2009). However, the strigolactone composition of the exudate may be a crucial factor here as it has been shown that there is a large difference in the capacity of individual strigolactones to induce hyphal branching in AM fungi (Akiyama et al. 2010). Rice varieties producing the right mix of strigolactones could have the desired tillering and AM-fungal phenotype while at the same time not inducing too much *Striga* germination. Since there is genetic variation for the strigolactone composition in rice, selection could potentially alter the strigolactone composition to a more desired mixture.

In conclusion, the tillering potential of rice turns out to be an important marker for the plant's susceptibility to *Striga* infection. This phenomenon could be helpful in making decisions about the extent of *Striga* infection to be expected in germplasm collections and could hence guide breeders in selecting the right materials when breeding for *Striga* resistance. Selection of suitable, high-tillering cultivars (which we have now shown to produce less strigolactones) might be a useful strategy to reduce serious cereal losses by these noxious parasitic weeds.

Acknowledgements

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Suppl. Table S1 Genotypic diversity of rice for tillering, *Striga* germination, emergence and dry biomass production

	Cultivars	Tillers		Germination		Attachment		Emergence		Dry biomass	
		(No. plant^{-1})	Rank[1]	(%)	Rank	(No. plant^{-1})	Rank	(No. plant^{-1})	Rank	(mg)	Rank
1	IAC 165	6±0 [a]	20	68±3	20	9±3	20	34±4	20	4680±296	20
2	IAC 1246	6±0.3	20	65±4	19	6±3	19	29±5	19	2903±229	19
3	Gangweondo	7±0.3	17	61±2	18	5±1	14	28±8	18	2523±212	17
4	Kinko	7±0.6	17	54±1	17	5±2	14	28±6	17	2626±96	18
5	Dullo	8±0	12	47±5	16	4±0	13	25±1	16	1833±120	13
6	Binagimbing	8±0	12	44±2	14	3±0	6	19±1	15	2167±176	16
7	Sonkanoir	8±0.3	12	43±2	12	3±1	6	17±3	12	1967±33	15
8	20 D	9±0.3	16	44±4	13	3±1	6	17±3	12	1700±153	12
9	TOS 7556	8±0.3	12	45±2	15	3±1	6	16±2	10	1900±100	14
10	Kairyo-HM	9±0	11	26±0	11	3±1	6	17±2	12	1338±324	11
11	PI 160641	11±0.6	9	20±4	9	3±1	6	11±4	9	732±132	9
12	Koirao Baleo	11±0.3	9	24±1	10	3±1	6	16±2	10	1012±149	10
13	Bhasmanik	12±0.3	5	18±4	7	3±0	6	9±3	6	325±78	5
14	S. -Chiang	12±0.3	5	19±1	8	3±1	6	9±3	6	533±88	7
15	Peh-kuh	13±0.6	4	16±2	5	3±1	6	9±2	6	592±64	8
16	Tattare	12±0.3	5	16±3	5	2±0	2	8±2	5	467±33	6
17	Agee	12±0.3	5	11±1	3	1±1	1	1±1	1	18±12	1
18	Anakila	14±0.3	3	12±1	4	2±1	2	1±1	1	19±19	2
19	TN 1	16±0.6	2	10±2	2	2±0	2	4±1	3	226±96	4
20	Super Basmati	18±0.6	1	9±1	1	2±1	2	4±1	3	37±25	3
	LSD 5%	1.0		7.5		3.4		6.8		431.9	
	P	<0.001		<0.001		<0.014		<0.001		<0.001	

[1]Ranking has done from 'resistance' to 'susceptible' with rank 1 to the best performing resistance genotype against *S. hermonthica* infection and 20 for the most susceptible one.

Suppl. Table S2 Genotypic diversity of rice for strigolactones production in root exudates

	Cultivars	2'-epi-5-DS		orobanchol		meth-5-DS-isomer 1		meth-5-DS-isomer 2		meth-5-DS-isomer 3	
		(PA 10^3)	Rank[1]	(PA 10^3)	Rank	(PA 10^3)	Rank	(PA 10^3)	Rank	(PA 10^3)	Rank
1	IAC 165	177.2 [a]	19	131.2[a]	20	6.4	20	158.5	20	152.3	20
2	IAC 1246	124.8	17	127.5	19	5.9	19	112.3	19	131.5	19
3	Gangweondo	201.8	20	20.3	14	3.7	17	87.0	18	126.2	18
4	Kinko	133.7	18	109.1	17	1.7	16	52.1	16	118.4	17
5	Dullo	47.5	16	125.0	18	0.7	13	20.5	14	37.0	14
6	Binagimbing	45.0	15	37.0	15	3.8	18	77.2	17	65.1	16
7	Sonkanoir	44.2	14	61.5	16	1.0	15	36.6	15	53.7	15
8	20 D	17.5	13	10.9	13	0.8	14	4.6	13	7.3	13
9	TOS 7556	5.7	11	5.1	12	0.2	2	2.7	12	4.5	12
10	Kairyo-HM	7.4	12	1.9	11	0.2	2	1.6	11	3.7	11
11	PI 160641	0.6	8	1.3	10	0.4	9	0.3	10	0.1	2
12	Koirao Baleo	0.5	6	0.8	6	0.4	9	0.2	4	0.6	10
13	Bhasmanik	1.4	10	0.4	2	0.2	2	0.2	4	0.1	2
14	Shuang-Chiang	0.4	2	0.9	8	0.3	7	0.1	1	0.2	5
15	Pehkuh	0.5	7	1.3	9	0.5	12	0.2	4	0.1	2
16	Tattare	1.0	9	0.8	7	0.1	1	0.1	1	0.2	5
17	Agee	0.4	2	0.6	4	0.2	2	0.2	4	0.3	7
18	Anakila	0.5	5	0.5	4	0.2	2	0.1	1	0.0	1
19	TN 1	0.4	4	0.4	2	0.3	7	0.2	4	0.3	7
20	Super Basmati	0.1	1	0.3	1	0.4	9	0.2	4	0.3	7
	LSD 5%	52.4		73.4		2.5		44.2		48.2	
	P	<0.001		<0.001		<0.001		<0.001		<0.001	

[a.]Means (*n*=3) [LSD]Least significant differences of means at $P = 0.05$ by ANOVA test.; [1]Ranking was done from 'high strigolactone producer' to 'low strigolactone producer' with rank 1 indicating the least producing genotype and 20 the highest producing one; PA: peak area (in 10^3); DS: deoxystrigol; meth: methoxy.

Suppl. Table S3 Genotypic diversity of rice for strigolactones production in root tissues

	Cultivars	2'-epi-5-deoxystrigol		orobanchol		meth-5-DS-isomer 1		meth-5-DS-isomer 2		meth-5-DS-isomer 3	
		(PA)	Rank	(PA)	Rank[1]	(PA)	Rank	(PA)	Rank	(PA)	Rank
1	IAC 165	2887.8 [a]	8	239.9 [a]	6	3.8	3	1.4	6	714.4	8
2	IAC 1246	2397.9	7	325.4	8	9.0	6	3.0	8	383.9	7
3	Gangweondo	296.7	5	2.5	4	6.8	4	1.0	4	2.4	5
4	Kinko	998.6	6	293.6	7	10.5	8	1.0	4	169.3	6
5	Agee	3.6	3	1.6	2	8.4	5	0.3	1	0.5	1
6	Anakila	2.1	2	2.6	2	3.7	2	1.6	7	0.6	2
7	TN 1	5.2	4	1.2	1	1.7	1	0.9	2	0.8	3
8	Super Basmati	1.5	1	4.4	5	9.0	6	0.9	2	0.8	3
	LSD 5%	61.2		51.2		4.1		1.2		72.1	
	P	<0.001		<0.001		<0.001		<0.001		<0.001	

[a.]Means (n=3) [LSD]Least significant differences of means at $P = 0.05$ by ANOVA test.; PA: peak area (in 10^3); [1]Ranking was done from 'high strigolactone producer' to 'low strigolactone producer' with rank 1 indicating the least strigolactone producing genotype and 8 the highest producing one; DS: deoxystrigol; meth: methoxy.

Suppl. Table S4 Contribution of individual strigolactones to the explanation of the variation in *Striga* germination, attachment and emergence

	Germination[1]	Attachment	Emergence	Tillers per plant
2'-epi-5-deoxystrigol	$*^{(+)}$	NS	$**^{(+)}$	$**^{(-)}$
orobanchol	NS	NS	NS	NS
methoxy-5-deoxystrigol isomer 1	NS	NS	NS	NS
methoxy-5-deoxystrigol isomer 2	NS	NS	NS	NS
methoxy-5-deoxystrigol isomer 3	$**^{(+)}$	NS	$**^{(+)}$	NS

[1]Germination of pre-conditioned *S. hermonthica* seeds in root exudates collected from various rice cultivars; attachment, emergence and tillering trend was studied in pot experiments. See Materials and methods for experimental details; $^{**}P$ <0.01; [NS]: Non-significant; Linear models were fitted to explain *S. hermonthica* germination (after logit transformation) with the individual strigolactones. Generalized linear models with Poisson distribution error and a log link were used to analyze *S. hermonthica* attachment with the individual strigolactones (known and unknown). $^{(+)}$: Positively associated; $^{(-)}$: Negatively associated

Suppl. Table S5 Ratios between orobanchol and 2'-epi-5deoxystrigol, methoxy-5-deoxystrigol isomer, and methoxy-5-deoxystrigol isomer 3 in root exudates and root tissu extracts

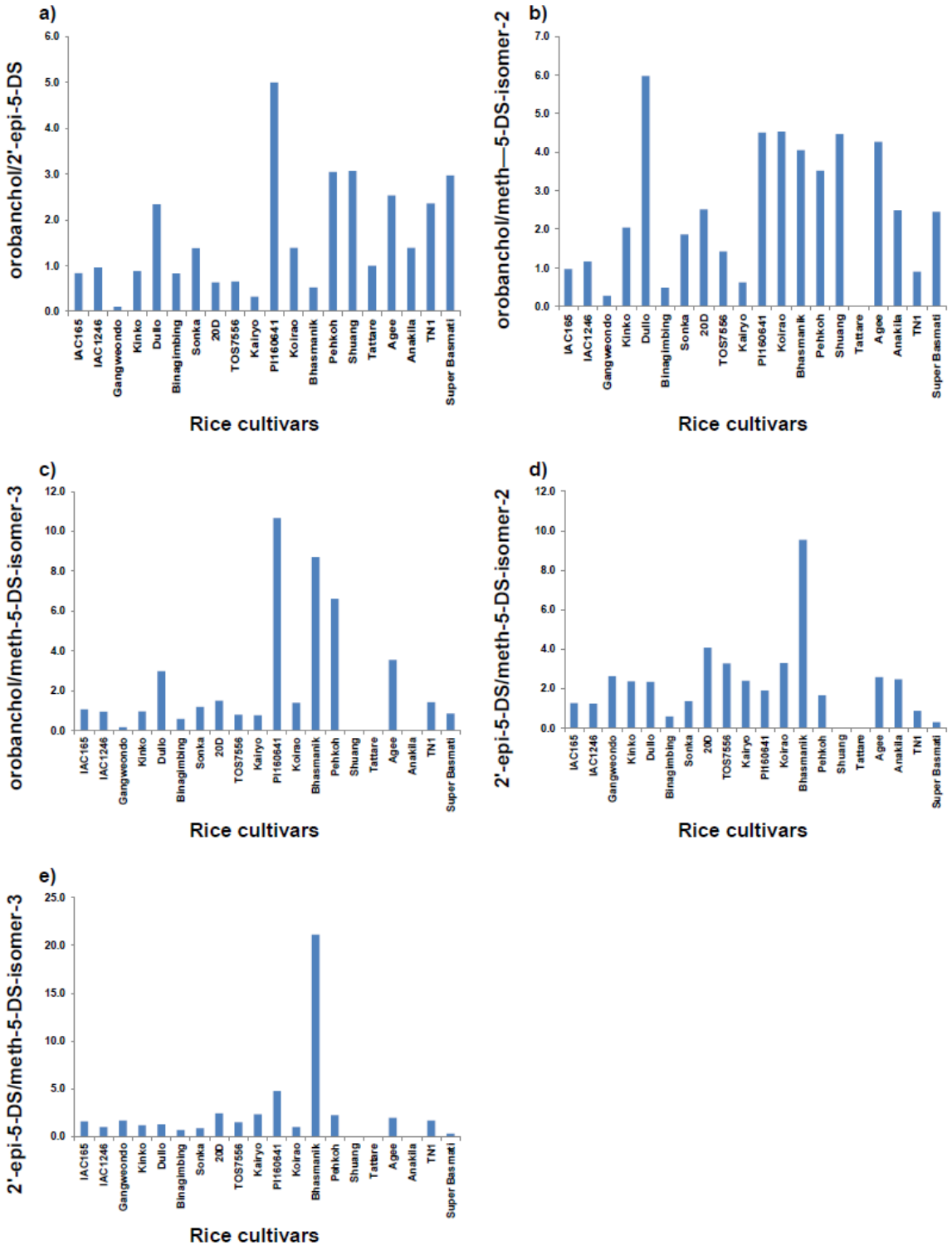
	Ratio among strigolactones collected from root exudates of 20 rice cultivars					Ratio among strigolactones collected from root tissue of 4 low tillering and 4 high tillering rice cultivars				
Cultivars	oro/ 2'-epi	Oro/ meth 2	oro/ meth 3	2'-epi / meth 2	2'-epi / meth 3	oro/ 2'-epi	oro/ meth 2	oro/ meth 3	2'-epi / meth 2	2'-epi / meth 3
IAC 165	0.8	1.0	1.1	1.3	1.6	0.08	223.01	0.34	2536.42	4.26
IAC 1246	1.0	1.2	0.9	1.2	1.0	0.13	446.53	2.53	3493.83	19.97
Gangweondo	0.1	0.3	0.2	2.6	1.7	0.20	175.88	1.55	774.34	24.71
Kinko	0.9	2.0	1.0	2.4	1.2	0.10	265.20	1.81	1095.59	286.13
Dullo	2.3	6.0	3.0	2.3	1.3					
Binagimbing	0.8	0.5	0.6	0.6	0.7					
Sonkanoir	1.4	1.9	1.2	1.4	0.9					
20 D	0.6	2.5	1.5	4.1	2.4					
TOS 7556	0.7	1.4	0.8	3.3	1.5					
Kairyo-HM	0.3	0.6	0.8	2.4	2.3					
PI 160641	5.0	4.5	10.7	1.9	4.8					
Koirao Baleo	1.4	4.5	1.4	3.3	1.0					
Bhasmanik	0.5	4.0	8.7	9.5	21.2					
Shuang-Chiang	3.0	3.5	6.6	1.7	2.3					
Pehkuh	3.1	4.5	0.0	0.0	0.0					
Tattare	1.0	0.0	0.0	0.0	0.0					
Agee	2.5	4.3	3.5	2.6	2.0	15.87	2.17	3.27	0.22	4.75
Anakila	1.4	2.5	0.0	2.5	0.0	3.13	1.57	4.65	0.48	3.42
TN 1	2.4	0.9	1.4	0.9	1.7	0.65	1.46	1.47	5.54	6.68
Super Basmati	3.0	2.5	0.9	0.3	0.3	7.96	49.00	6.51	3.94	6.93
Mean	1.6	2.4	2.2	2.2	2.4	3.5	145.6	2.8	988.8	44.6
S.D.	1.2	1.7	3.0	2.1	4.6	5.8	169.6	1.4	1385.0	104.5
C.V.	77.0	71.7	135.0	92.7	190.4	165.9	116.5	50.7	140.1	234.2

oro: orobanchol; meth: methoxy; S.D.: standard deviation; C.V.: coefficient of variation

Suppl. Table S6 Relationship between various strigolactones and *Striga* germination, attachment, emergence and dry biomass production in low and high tillering rice cultivars

	Correlation coefficient r, by trait number							
Trait no. and description	1	2	3	4	5	6	7	8
1. orobanchol								
2. 2'-epi-5-deoxystrigol	0.63**							
3. methoxy-5-deoxystrigol-isomer 1	0.60**	0.77**						
4. methoxy-5- deoxystrigol-isomer 2	0.64**	0.81**	0.95**					
5. methoxy-5- deoxystrigol isomer 3	0.66**	0.81**	0.82**	0.92**				
6. Germination	0.67**	0.78**	0.68**	0.76**	0.81**			
7. Attachment	0.42*	0.54*	0.52*	0.62**	0.62**	0.57*		
8. Emergence	0.61**	0.75**	0.68**	0.75**	0.77**	0.87**	0.67**	

*P <0.05; **P <0.01; NS: Non-significant; n=3 r = Correlation coefficient



Suppl. Fig. 1a-e Ratio between strigolactones orobanchol, 2'-epi-5-deoxystrigol and methoxy-5-deoxystrigol isomers 2 and 3 in the root exudates of various rice cultivars

Chapter 5

Quantification of the relationship between strigolactones and *Striga hermonthica* infection in rice under varying levels of nitrogen and phosphorus

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Abstract

Strigolactone exudation, as well as *Striga hermonthica* (*Striga*) germination and attachment, were studied under different levels of nitrogen (N) and phosphorus (P) in two cultivars of rice (IAC 165 and TN 1). Exudation of strigolactones by rice was the highest under mineral deficient conditions, whereas increasing N and P dose reduced the amount of strigolactones in the exudates. Deficiency of P led to the highest strigolactone exudation, as compared with N or NP deficiency. Production of strigolactones differed strongly between the two cultivars. IAC 165 produced about 100-fold higher amounts than TN 1 of 2'-epi-5-deoxystrigol, orobanchol and three new strigolactones. Across all N and P treatments, a positive relationship was found between the amount of strigolactones in the exudates of both cultivars and *in vitro Striga* germination. These results show that the positive effect of fertilizer application in *Striga* control is, at least partly, due to the suppression of strigolactone production and hence of *Striga* germination and subsequent attachment. This warrants further research into practical application. Maintaining suitable N and P nutrient status of soil through fertilizer use might be a promising strategy to reduce damage in cereals by this notorious weed.

Keywords: *Striga hermonthica*, strigolactones, nitrogen, phosphorus, germination, parasitic weed, attachment.

Introduction

Rice (*Oryza glaberrima* S.; *Oryza sativa* L.) is an increasingly important food source in 52 countries of Sub-Saharan Africa. Due to higher consumption, the demand for rice in the African continent has increased by almost 6% per year during the past two decades (WARDA 2005; Rodenburg and Johnson 2009). The root parasitic weeds *Striga hermonthica* (Del.) Benth and *Striga Asiatic* (L.) Kuntze are among the most serious biotic constraints in the African continent, where they threaten production of important cereals such as sorghum, maize and millet. Because of their ability to infect cereals, they are also becoming a serious threat for rice production. The infestation by these parasitic weeds is one of the main causes of low yields in the African continent, where they parasitize roots of upland rice in semi-arid to sub-humid tropics (Harahap et al. 1993) and damage is reported to range from 25-50% with total crop loss in extreme cases (Johnson et al. 1997).

Striga spp. have been described as indicators of low soil fertility and their infestation is closely linked with low nutrient conditions (Oswald 2005). Reduction in *Striga* spp. and improvement in crop yields through the use of mineral nutrients have been reported in numerous studies (Smaling et al. 1991; Kim and Adetimirin 1997; Ransom 2000; Gacheru and Rao 2001). It has been suggested that soil fertility and mineral nutrients not only stimulate growth of the host, but also adversely affect germination, attachment and subsequent development of the weed (Abunyewa and Padi 2003). Direct inhibition of *Striga* spp. seed germination by nitrogen (N) and phosphorus (P) fertilization has been suggested in many studies (Raju et al. 1990; Gworgwor and Weber 1991; Cechin and Press 1993;). Increasing nitrogen content and asparagin formation due to excessive accumulation of nitrogen (as NO_3^-, NO_2^{2-} and NH_4^+) by nitrogen fertilization in the roots of sorghum resulted in the death of *Striga* spp. (Simier et al. 2006). Another indicator of soil fertility, the presence of organic matter, not only increased the soil's ability to suppress *Striga* spp. directly but also caused suicidal germination through ethylene production by enhancing microbial activity (Ahonsi et al. 2003; Sauerborn et al. 2003). Soil fertility improvement through intercropping and crop rotation proved to suppress *Striga* spp. possibly by influencing microbial activity, shading, allelopathy and suicidal germination (Carsky et al. 1994; Khan et al. 2002; Abunyewa and Padi 2003; Reda et al. 2005). In conclusion, it is clear that improvement of soil fertility can decrease the *Striga* spp. problem. However, even though many possible explanations have been given for the *Striga* spp. suppressing effect of soil fertility, the exact mechanism by which fertilizer works to suppress *Striga* spp. is unknown.

One of the many mechanisms by which plants will try to overcome nutrient deficiency is to engage in symbiosis with arbuscular mycorrhizal (AM) fungi (Akiyama et al. 2005). Under nutrient deficiency, plants activate AM fungi through underground communication by releasing signalling molecules. Upon activation by these molecules, the strigolactones, a symbiotic relationship is established in which the AM fungi facilitate mineral nutrient uptake by the host, particularly of

phosphorus and nitrogen, in return for carbohydrates (Harrison 2005). Upon strigolactone perception, AM fungi engage in hyphal branching, a process that improves the host root colonization success rate. Strigolactones are hence a crucial factor in the establishment of this symbiosis, but at the same time the germination of parasitic weed seeds is induced by these same signalling compounds (Bouwmeester et al. 2003; Akiyama et al. 2005; Bouwmeester et al. 2007). In line with the importance of AM fungi for the uptake of mineral nutrients, it has been demonstrated that strigolactone exudation is increased under mineral nutrient deficiency, particularly of phosphorus (Yoneyama et al. 2007a, b; Lopez-Raez et al. 2008).

In the present paper, we study the mechanism responsible for reduced *Striga* spp. parasitism with fertilizer application. Our hypothesis was that a better mineral nutrient supply affects *Striga* spp. infection indirectly by reducing the exudation of the strigolactones into the rhizosphere. To test this hypothesis, experiments were carried out under controlled conditions to quantify the relationship between strigolactone exudation and *Striga* spp. germination and attachment under different levels of nitrogen and phosphorous, using rice as a model host crop.

Materials and methods

Seeds

Seeds of rice cultivar (cv.) IAC 165 were obtained from the University of Sheffield, UK (courtesy of Prof. Julie Scholes) and seeds of cv. TN 1 from the International Rice Research Institute, Philippines (courtesy of Ms. Flora De Guzman). *Striga hermonthica* seeds used in the attachment study were collected from a sorghum field near Cinzana, Mali (courtesy of Cheickna Diarra) and *Striga* seeds used in germination bioassays with rice root exudates were collected from a sorghum field near Wad Medani, Sudan (courtesy of Prof. A.G. Babiker).

Chemicals

The mineral nutrients used in Hoagland's nutrient solution (NH_4NO_3, KNO_3, K_2HPO_4, $FeSO_4.7H_2O$, Na_2EDTA, $CaCl_2$, K_2SO_4, $MgSO_4.7H_2O$) were obtained from Merck and Fluka, Germany and Duchefa, the Netherlands. The synthetic strigolactone, GR24, was provided by Binne Zwanenburg (Department of Organic Chemistry, Radboud University, Nijmegen, the Netherlands). Standards of orobanchol, 2'-epi-orobanchol and 5-deoxystrigol were provided by Koichi Yoneyama (Weed Science Center, Utsunomiya University, Japan) and 2'-epi-5-deoxystrigol by Kohki Akiyama (Osaka Prefecture University, Japan). D_6-2'-epi-5-deoxystrigol was synthesized as described (Ueno et al. 2010). SPE C18-Fast column (500 mg/3 mL) was purchased from Grace Davison Discovery Sciences, Belgium. The sterilizing reagent sodium hypochlorite was purchased locally (Van Dam Bodegraven) and Tween-20 was obtained from Merck, Germany.

Experimental details

Three sets of experiments were conducted under controlled conditions in Wageningen, the Netherlands. The experiments were laid out in completely randomized design (factorial) with three replications. Two varieties of rice (cvs IAC 165 and TN 1) were treated as main factors, while various levels (0%, 25%, 50% and 100%) of nitrogen (N) or phosphorus (P) or both (NP) were tested as sub-factors in each variety. The mineral nutrients were applied through half strength modified Hoagland's nutrient solution (250 mL per 48 hours) (Hoagland and Arnon 1950). For the different nitrogen levels, the amount of NH_4NO_3 and KNO_3 were reduced to 0%, 25% or 50% of the maximum amount (100%), while the rest of the nutrients remained unchanged. For the different phosphorus levels, the amount of K_2HPO_4 was reduced to 0%, 25% or 50% of the maximum amount (100%), while the rest of the nutrients remained unchanged. For the different NP levels, the amount of NH_4NO_3, KNO_3 and K_2HPO_4 were reduced to 0%, 25% or 50% of the maximum amount (100%), while the rest of the nutrients remained unchanged. In the reduced N, P and NP treatments, K was adjusted to half strength modified Hoagland level by adding K_2SO_4 stock solution.

Root exudate collection

Seeds of rice were surface sterilized in 25 mL (2%) sodium hypochlorite containing 0.4% Tween-20, rinsed thoroughly with sterile demineralized water and put to germinate on moistened filter paper at 30°C. After 48 hours, germinated seeds (20 rice seeds per pot) were planted in 1 L sand and grown for four weeks in a glasshouse at 28°C (10 h)/25°C (14 h) photoperiod and 70% relative humidity. Half-strength modified Hoagland's nutrient solution was applied at 250 mL per pot at 48 h intervals. Four weeks after planting, each pot was washed with 3 L of nutrient solution without N and P that was allowed to drain from the pot. After washing and draining, the pots were treated with various treatments (0, 25, 50 and 100% of N, P or NP) by applying and draining the surplus of 1 L nutrient solution containing the appropriate mineral nutrient concentration. The pots were watered as before with the appropriate nutrient solutions for one week and then pots were again washed with 2 L nutrient solution of the corresponding mineral nutrient composition to remove any accumulated exudates. Forty eight hours later, root exudates were collected in plastic bottles by draining the pots with 1 L of nutrient solution of the appropriate nutrient composition. These root exudates were then passed through a C18-Fast column (500 mg/3 mL) and the strigolactones eluted with 6 mL of 100% acetone. These samples were used for LC-MS/MS analysis and *Striga* germination bioassays.

***Striga* germination**

Root exudates obtained under different levels of mineral nutrients were assessed for germination stimulatory activity by germination bioassays with *Striga* seeds, as reported before (Matusova et al. 2005). For preconditioning, surface-sterilization of *Striga* seeds was done using 25 mL (2%) sodium

hypochlorite with 0.4% of Tween-20 for 5 minutes. Subsequently, seeds were thoroughly rinsed three times with 10 min intervals using sterile demineralized water through a Buchner funnel. The sterile seeds were air dried for sixty minutes. Approximately 50 to100 seeds were then evenly spread on 9-mm diameter glass fiber filter paper discs (Sartorius, Germany). These discs were placed in 9 cm diameter Petri dishes (12 discs per dish) on filter paper (Waterman, UK) moistened with 3 mL demineralized water. The Petri dishes were sealed with parafilm, wrapped in aluminium foil and placed in an incubator at 30ºC for 10 days.

After 10 days, the discs with preconditioned seeds were allowed to dry for 50 min in a laminar flow cabinet to evaporate surplus moisture. The discs were then placed in another Petri dish (six per dish) containing a filter paper ring (outer diameter 9 cm, inner diameter 8 cm) moistened with 0.9 mL water. The samples to be tested were applied (50 µL) to triplicate discs after replacement of the acetone in the samples by water through vacuum centrifugation. GR24 (3.3 µM) was used as a positive control and water as a negative control in each germination assays. Seeds were again incubated at 30ºC in darkness for 48 hours and germination (seeds with radicle protruding through the seed coat) was scored using a binocular microscope (Matusova et al. 2005).

Strigolactones analysis using liquid chromatography-tandem mass spectrometry

The root exudates of rice cvs IAC 165 and TN 1 were analysed by LC-MS/MS for the presence of strigolactones. For this, 3 mL of the acetone fractions after C18 purification obtained as described above were transferred to a glass vial and the acetone evaporated in a GYROVAP vacuum centrifuge. Subsequently, 300 µL of 25% acetonitrile was added and the samples mixed thoroughly using vortex. Each sample was then cleaned manually by syringe filter (Minisart SRP_4) and subjected to LC-MS/MS analysis. To quantify the amount of orobanchol and 2'-epi-5-deoxystrigol, 2 mL of the acetone fraction was taken in a glass vial and 0.02 nmol of D_6-2'-epi-5-deoxystrigol as internal standard was added to each sample. Finally, each sample was concentrated 10-fold.

The identification of strigolactones was done by comparing retention times and mass transitions with those of available strigolactone standards, such as orobanchol, 2'-epi-orobanchol, 5-deoxystrigol, 2'-epi-5-deoxystrigol, sorgolactone, strigol, solanacol and orobanchyl acetate, using ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS), as described by (Lopez-Raez et al. 2008). Analyses were performed using a Waters Micromass Quattro Premier XE tandem mass spectrometer (Waters, Milford, MA, USA) equipped with an ESI source and coupled to an Acquity UPLC system (Waters, USA). Chromatographic separation was achieved using an Acquity UPLC BEH C_{18} column (150 - 2.1 mm, 1.7 µM) (Waters, State, USA), applying a water/acetonitrile gradient, starting at 0% acetonitrile for 0.5 min, raised to 25% acetonitrile in 0.5 min, followed by a 6.5 min gradient to 40% acetonitrile, followed by a 4.5 min gradient to 65% acetonitrile, which was then maintained for 0.1 min and followed by a 0.1 min gradient back to 0%

acetonitrile before the next run. The column was equilibrated at this solvent composition for 2.05 min. Total run time was 14.25 min. The column was operated at 50°C with a flow-rate of 0.4 mL min^{-1} and the sample injection volume was 20 µL. The mass spectrometer was operated in positive electrospray ionization (ESI) mode. The nebulizer and desolvation gas flows were 50 and 800 Lh^{-1}, respectively. The capillary voltage was set at 2.7 kV, the cone voltage at 20 V, the source temperature at 120°C, and the desolvation gas temperature at 450°C. Fragmentation was performed by collision induced dissociation with argon at 0.36Pa $3.6x10^{-3}$ mbar. Multiple reaction monitoring (MRM) was used to search for strigolactones. The MRM transitions were set according to the MS/MS spectra obtained for the standards. Protonated molecular ions $[M + H]^+$ were the most abundant in the full-scan mass spectra obtained from the standard strigolactones. Therefore, they were selected as parent ions for the transitions. Two or three parent-daughter transitions were selected for each strigolactone, according to the most abundant and/or specific fragment ions for which the collision energy (CE) was optimized. For orobanchol and 2'-epi-orobanchol, the transitions (channels) selected were *m/z* 347>233 at 10eV, 347>205 at 15eV and 347>97 at 18eV; for 5-deoxystrigol and 2'-epi-5-deoxystrigol the transitions *m/z* 331>234, 331>217, 331>97 at the collision energy 10 and 18eV were selected. MRM transitions (channels) *m/z* 361>247 and 361>97 were used for three new strigolactones. Data acquisition and analysis were performed using Mass Lynx 4.1 software (Waters, USA). For each compound, the summed peak area of all the corresponding MRM transitions was used for statistical analysis.

For the quantification of the levels of orobanchol and 2'-epi-5-deoxystrigol in rice root exudates, the samples prepared with addition of D_6-2'-epi-5-deoxystrigol as internal standard were analyzed using a Waters Xevo TQ MS tandem mass spectrometer (Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) source and coupled to an Acquity UPLC system (Waters, USA). Chromatographic separation was achieved on an Acquity UPLC BEH C_{18} column (150 x 2.1 mm, 1.7 µm) (Waters) by applying a water/acetonitrile gradient to the column, starting from 5% acetonitrile for 0.5 min and rising to 27% acetonitrile at 1.0 min, followed by a 6.5 min gradient to 40% acetonitrile, and 4.5 min gradient to 65% acetonitrile, and maintained for 0.1 min, followed by a 0.4 min gradient to 90% acetonitrile, which was maintained for 0.2 min, before going back to 5% acetonitrile using a 0.3 min gradient, prior to the next run. Finally, the column was equilibrated for 3 min, using this solvent composition. The column was operated at 50°C with a flow-rate of 0.4 mL min^{-1}. Sample injection volume was 10 µL. The mass spectrometer was operated in positive ESI mode. Cone and desolvation gas flows were set to 50 and 1000 L h^{-1}, respectively. The capillary voltage was set at 3.0 *kV*, the source temperature at 150ºC and the desolvation temperature at 650ºC. The cone voltage was optimized for each strigolactone standard using the IntelliStart MS Console. Argon was used for fragmentation by collision induced dissociation (CID).

Multiple reaction monitoring was used for quantification of strigolactones in rice root exudates. MRM transitions were optimized for each standard compound using the IntelliStart MS

Console. For orobanchol the transitions *m/z* 347>233, 347>205 and 347>97 at the collision energy 12eV, 18eV and 22eV; for and 2'-epi-5-deoxystrigol the transitions *m/z* 331>234, 331>216 at the collision energy 10 and 15eV and for D_6-2'-epi-5-deoxystrigol the transitions *m/z* 337>240 and *m/z* 337>97 at the collision energy 10eV and 22eV were selected. The orobanchol and 2'-epi-5-deoxystrigol levels were quantified using a calibration curve with known amount of standards and based on the ratio of the peak areas of the MRM transitions for orobanchol and 2'-epi-5-deoxystrigol to those for D_6-2'-epi-5-deoxystrigol. Data acquisition and analysis were performed using Mass Lynx 4.1 (TargetLynx) software (Waters).

***Striga* attachment**

About 20 mg (4000) *Striga* seeds were weighed for each treatment and mixed thoroughly in 500 mL washed river sand. Plastic pots (16 cm diameter x 13 cm height) were used with a sheet of perforated plastic foil placed in the bottom of the pot to avoid sand washing out of the pot through the drainage holes. About 100 mL of sand without *Striga* seeds was placed in the bottom of the pot. Then 500 mL of sand with 20 mg *Striga* seed were added on top. Rice seeds were surface sterilized and germinated as described above. One germinated seed was planted in the middle of each pot. The seedlings were allowed to grow in a glasshouse at 28°C day (10 h) / 25°C night (14 h) with 70% relative humidity. The appropriate mineral nutrient levels were created using half strength modified Hoagland's nutrient solution. Eight weeks after planting, *Striga* emergence was scored. Subsequently, the sand was carefully removed from the plant roots and the number of *Striga* attachments counted using a stereomicroscope.

Data collection and analysis

Data were subjected to analysis of variance (ANOVA) to determine treatment effects by using GenStat Release 9.2 (VSN International Ltd, UK). A linear regression model was fitted to correlate *Striga* germination (after logit transformation) to the different known and new strigolactones. A Poisson Generalized Linear Model (GLM) was fitted to correlate *Striga* attachment (No. per plant) to the different strigolactones. To select the strigolactones that are important for *Striga* germination and for attachment in two rice cultivar, a stepwise algorithm based on Akaike Information Criterion (AIC) was used (Akaike 1981).

Results

***Striga* germination**

The application of root exudates collected at various levels of mineral nutrients caused germination of pre-conditioned *Striga* seeds (Table 1). Root exudates collected under P deficiency (0% P) resulted in

the highest *Striga* germination in both rice cvs IAC 165 and TN 1, while increasing P levels led to progressively lower germination. TN 1 overall induced less germination (about half) than IAC 165. Also for N and NP, maximum *Striga* germination was induced in both cultivars by root exudates of plants treated with 0 or 25% N/NP, while higher levels of N and NP led to lower germination percentages.

Table 1 Effect of varying levels of nitrogen (N), phosphorus (P) or both (NP) on *Striga* germination and attachment. The synthetic strigolactone GR24 at 3.3 µM induced 52% germination

	Germination (%)		Attachment (No. per plant)		Attachment (No. per gram of root dry biomass)	
	IAC 165	TN 1	IAC 165	TN 1	IAC 165	TN 1
Nitrogen (N) levels						
0%	10.9[a]±1.7	8.1[a] ±0.5	12.3 [a] ±2.4	4.0 [a] ±0.6	63.4 [a]±15.6	6.2 [a] ±0.7
25%	16.3±3.2	7.8±1.8	16.0±1.7	7.0±1.0	11.2±0.6	2.0±0.3
50%	7.1±1.5	4.5±0.8	2.3±0.3	1.0±0.0	0.9±0.2	0.3±0
100%	1.0±0.0	1.1±0.0	1.0±0.0	0.7±0.3	0.4±0	0.2±0.1
LSD (5%)	3.3		2.4		11.7	
Phosphorus (P) levels						
0%	29.5±5.1	15.3±2.5	19.0±1.7	5.7±1.7	50.1±11.6	9.4±4.8
25%	13.8±1.3	7.4±1.2	10.3±0.9	2.3±0.3	8.0±1.8	0.8±0.1
50%	7.5±1.1	5.2±0.6	2.7±0.3	1.3±0.3	1.1±0.1	0.5±0.1
100%	1.2±0.1	1.0±0	1.0±0.0	0.7±0.3	0.4±0	0.2±0.1
LSD (5%)	4.5		2.0		9.5	
Nitrogen & Phosphorus (NP) levels						
0%	18.7±0.8	8.7±1.0	10.7±0.9	5.7±0.3	44.0±3.2	10.2±1.0
25%	13.9±5.3	5.8±0.7	7.0±0.6	5.7±0.3	4.5±0.2	1.7±0.2
50%	6.5±1.4	4.0±0.9	4.3±0.9	1.3±0.3	2.3±0.4	0.4±0.1
100%	1.3±0.2	1.0±0	0.7±0.3	0.7±0.3	0.3±0.1	0.2±0.1
LSD (5%)	4.3		1.2		2.5	

[*]*P* <0.05; [**]*P* <0.01; [a.]Means±standard error n=3

Striga emergence and attachment

Deficiency of P significantly increased *Striga* attachment in both rice cultivars. *A*ttachment was 2- to 4-fold higher in IAC 165 than in TN 1 (Table 1). The infection of *Striga* was highest under P deficiency and decreased with increasing P levels. Nitrogen, as well as NP deficiencies (0-25%), also caused

higher *Striga* attachment in both rice cultivars, while increasing levels of these nutrients reduced the attachment. To correct for a possible effect of differences in plant size, the number of *Striga* attachments per gram root dry weight was also calculated. Attachment was 4- to 10-fold higher in IAC 165 than TN 1 (Table 1). Maximum *Striga* infection per gram dry root biomass was found under N deficient conditions in cv. IAC 165, while higher levels of N caused lowest number of infection (Table 1). A similar trend was also found in TN 1, but with lower numbers than IAC 165. P, as well as NP deficiency, also induced the highest *Striga* infection per gram root dry weight in both cultivars, decreasing gradually to a minimum with increasing P and NP levels.

Strigolactone exudation

In the LC-MS/MS chromatograms, five peaks were detected that were present across a number of the different MRM channels. Comparison of the *R*t and corresponding mass transitions with those of authentic standards showed that the compound eluting at 8.08 min and detected in MRM channels *m/z* 347>233, 347>205, 347>97 is orobanchol (Fig. 1A); the compound eluting at 12.57 min and detected in MRM channels *m/z* 331>234, 331>217 and 331>97 is 2'-epi-5-deoxystrigol (Xie et al. 2007) (Fig. 1A). Three compounds - eluting at 9.90, 10.37 and 10.90 min and detected in MRM channels *m/z* 361>247 and 361>97 - are new strigolactones of which the identification is in progress (data not shown). The presence of orobanchol and 2'-epi-5-deoxystrigol in root exudates of rice plants was further confirmed by their MS/MS fragmentation spectra (Fig. 1B & 1C) and standard addition experiments. Standard addition of orobanchol and 2'-epi-5-deoxystrigol to the samples also confirmed that the observed reduction in peak areas in the root exudates from both cultivars under varying levels of mineral nutrients is real and not caused by differences in ion suppression during ionization in the ion source of the mass spectrometer (data not shown).

Rice cv. IAC 165 produced much higher amounts of 2'-epi-5-deoxystrigol, orobanchol and the three new strigolactones than TN 1. The peak area of 2'-epi-5-deoxystrigol, orobanchol and the three new strigolactones were about 100-fold higher ($P < 0.01$) in cv. IAC 165 than TN 1 (Fig. 2 A-J). Deficiency of P strongly stimulated strigolactone exudation. A smaller effect on strigolactone exudation was observed under N and NP starvation, but generally it was also highest under low levels and decreased with increasing N and NP levels. The amounts of known strigolactones 2'-epi-5-deoxystrigol and orobanchol were quantified in IAC 165 (amounts in TN 1 were too close to the detection level for reliable quantification) using a D-labeled internal standard. Maximum exudation of strigolactones, at 734 pmol/20 plants for 2'-epi-5-deoxystrigol and 573 pmol/20 plants for orobanchol, occurred under P deficiency and decreased with increasing P to undetectable at 100% P (Table 2). Production levels under N and NP starvation were much lower than under P starvation alone, at 40 and 85 pmol/20 plants for 2'-epi-5-deoxystrigol and orobanchol, respectively, under 0% N and 59 and 109 pmol/20 plants for 2'-epi-5-deoxystrigol and orobanchol, respectively, under 0% NP.

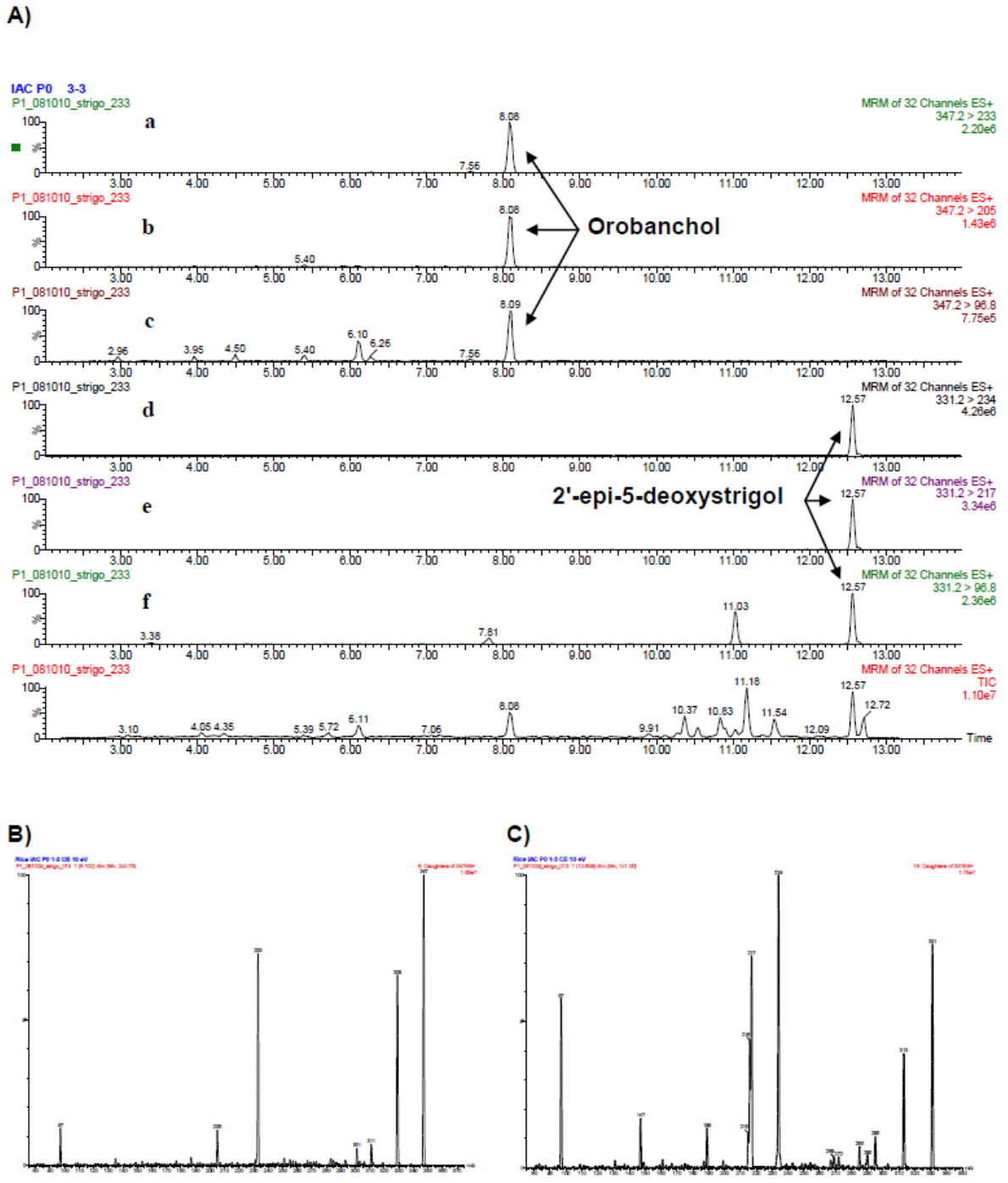

Fig. 1 LC/MS/MS analysis using multiple reaction monitoring (MRM) of rice root exudates. The MRM transitions for orobanchol (a, b, c) and 2'-epi-5-deoxystrigol (d, e, f) obtained for rice cv. IAC 165 root exudates are shown as example (A). MS/MS fragmentation spectra of the protonated molecular ions of orobanchol (B) and 2'-epi-5-deoxystrigol (C) are shown. The MS/MS spectra were recorded during online separation of rice cv. IAC 165 root exudates.

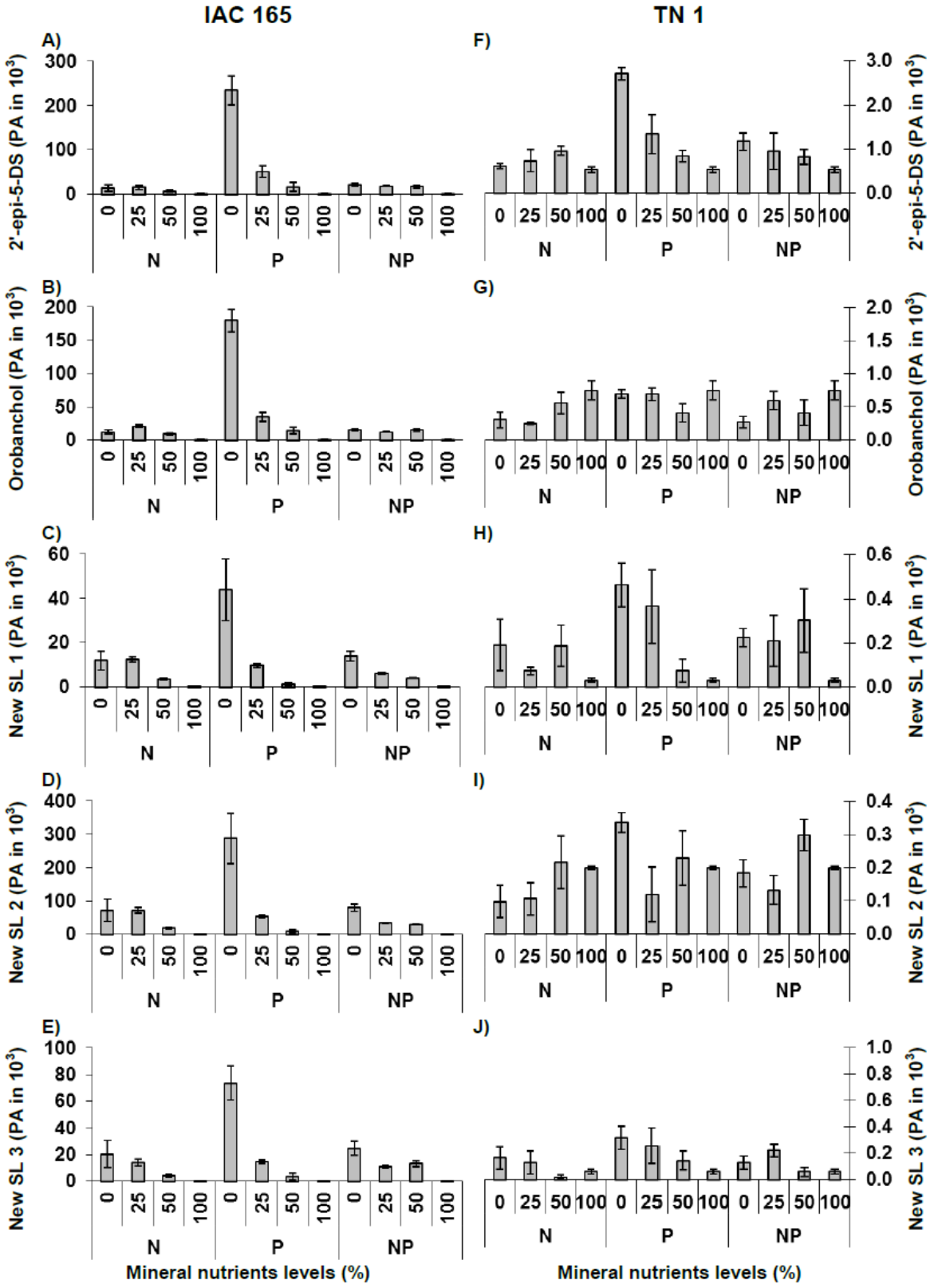

Fig. 2 Peak area (PA) of 2'-epi-5-deoxystrigol, orobanchol, new strigolactones (SL) 1-3 under varying levels of nitrogen (N), phosphorus (P) and both (NP) in the root exudates of *Oryza sativa* cultivar IAC 165 (A-E) and TN 1 (F-J). Bars represent means ± SE (n=3). Note the difference in scale on the y-axes

Correlation between strigolactones and *Striga* germination and attachment

The amount of strigolactones in the exudates and *Striga* germination across different levels of mineral nutrients in IAC 165 were positively correlated (Fig. 3 A-J), while in TN1 only for 2'-epi-5-

Table 2 Quantification of strigolactones in susceptible rice cultivar IAC 165

	2'-epi-5-deoxystrigol (pmol 20 plants^{-1} 48h^{-1})	orobanchol (pmol 20 plants^{-1} 48h^{-1})
Nitrogen (N) levels		
0%	40.2 [a] ±13.2	84.5 [a] ±24.3
25%	83.5±18.0	225.7±37.2
50%	85.4±59.2	124.8±56.5
100%	0.0±0	0.0±0
LSD (5%)	-	84.6
P	0.158	0.004
Phosphorus (P) levels		
0%	734.3 [a] ±51.6	573.3 [a] ±26.8
25%	102.0±19.4	281.4±111.2
50%	35.8±18.7	90.9±61.0
100%	0.0±0	0.0±0
LSD (5%)	90.6	246.4
P	<.001	0.005
Nitrogen & Phosphorus (NP) levels		
0%	58.7 [a] ±5.2	108.8 [a] ±19.7
25%	41.8±3.0	83.3±4.9
50%	31.0±3.4	78.3±11.9
100%	0.0±0	0.0±0
LSD (5%)	9.3	38.0
P	<.001	0.002

$^{*}P$ <0.05; $^{**}P$ <0.01; [a.] Means ± standard error n=3

The levels of the strigolactones orobanchol and 2'-epi-5-deoxystrigol in the root exudates of rice cv. IAC 165 were quantified by adding D_6-2'-epi-5-deoxystrigol as internal standard and using a Waters Xevo TQ MS tandem mass spectrometer (Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) source and coupled to an Acquity UPLC system (Waters, USA) for analysis.

Table 3 Strigolactone contribution to *Striga* germination and attachment

	Germination		Attachment	
	IAC 165	TN1	IAC 165	TN 1
2'-epi-5-deoxystrigol	NS	*	NS	NS
orobanchol	NS	**	NS	NS
new strigolactone-1	*	NS	***	NS
new strigolactone-2	*	NS	***	NS
new strigolactone-3	*	NS	***	*

$^{*}P$ <0.05; $^{**}P$ <0.01; $^{***}P$ <0.001; NS: Non-significant; Linear regression models were fitted to relate *Striga* germination (logit transformation) with best combination of strigolactones (known and new). Generalized linear model for Poisson distribution with logarithmic link was applied to relate strigolactones (known and new) to *Striga* attachment.

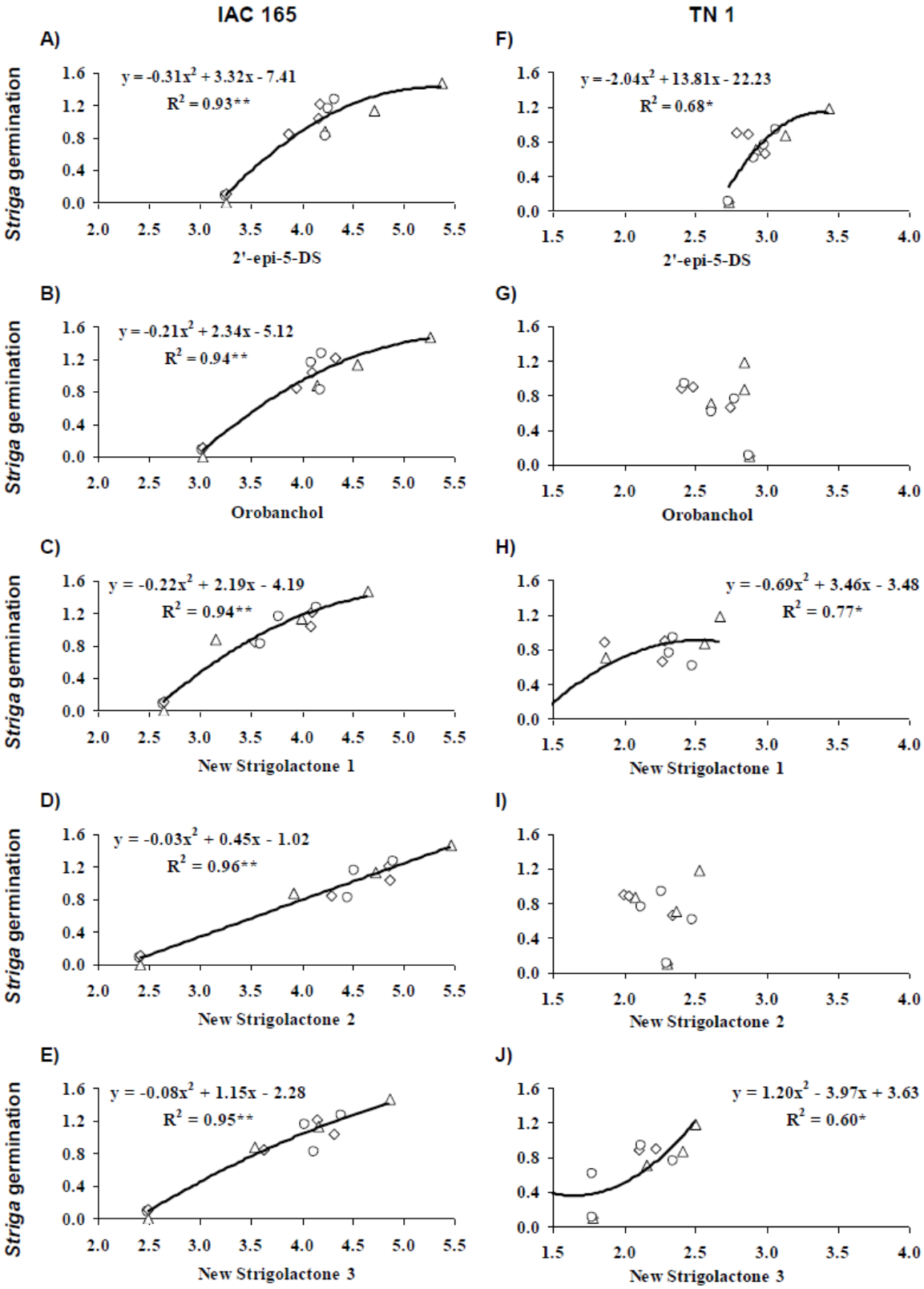

Fig. 3 Relationship between strigolactones and *Striga* germination under varying levels of N (◊), P (Δ) and NP (○) in cv. IAC 165 (A-E) and cv. TN 1 (F-J). The peak area of 2'-epi-5-deoxystrigol (DS), orobanchol and new strigolactones 1-3 were log transformed and associated with each other by regression analysis. ($^{**}P$ <0.01; $^{*}P$ <0.05).

deoxystrigol there was a significant correlation, probably due to the low concentrations of strigolactones in TN1 (Fig. 3 A-J). Regression analysis on the two known and three new strigolactones revealed that the three new strigolactones contributed significantly to the explanation of the variation in *Striga* germination in IAC 165 (Table 3). The three new strigolactones also contributed significantly to the explanation of variation in *Striga* attachment in IAC 165 (Table 3). In TN 1, 2'-epi-5-deoxstrigol and orobanchol contributed significantly to the explanation of the variation in *Striga* germination whereas for *Striga* attachment, new strigolactone 3 best explained the variation.

Discussion

Under nutrient deficient conditions, the secretion of signalling molecules, the strigolactones, is a natural phenomenon in plants that stimulates AM fungi to overcome nutrient deficiency through symbiosis. The seeds of parasitic weeds such as *Striga* and *S. asiatica*, however, also perceive these signalling molecules. In response, they germinate, attach to the host root and start parasitizing the host plant. In the present paper, we clearly demonstrate that the amount of strigolactones exuded by rice roots depends on the level of mineral nutrients in the soil. In addition, there is a clear correlation between the amount of strigolactones secreted by the host and the germination and subsequent attachment of *Striga*. Hence, the soil nutritional status, strigolactones exudation and infection are closely interrelated.

A decrease in *Striga* infection by the application of N and P has been reported in a number of studies (Raju et al. 1990; Smaling et al. 1991; Kim and Adetimirin 1997; Simier et al. 2006;). Moreover, a direct or indirect relationship between the presence of mineral nutrients and germination of *Striga* spp. has also been suggested by several authors (Cechin and Press 1993; Gacheru and Rao 2001; Kamara et al. 2007). In other studies, a direct inhibition of parasitic weed seed germination by N and P fertilizer was suggested (Raju et al. 1990)and also the presence of organic matter in the soil was shown to affect germination of *Striga* spp. (Sauerborn et al. 2003). In all these studies, there is a lack of consensus about the mechanism by which soil fertility reduces *Striga* infection. With the recent discovery that strigolactone exudation is affected by nutrient deficiency, it became clear that strigolactones secretion may be a crucial factor in explaining the effects of soil fertility on *Striga* spp. Infection (Yoneyama et al. 2007a, b; Lopez-Raez et al. 2008).

The present study also showed that exudation of strigolactones in rice strongly depended on the levels of mineral nutrients (Fig. 2 A-J). The lower the availability of N and particularly P, the higher the secretion of strigolactones. This higher secretion of strigolactones correlated closely with an increased germination in a bioassay (Fig. 3 A-J; Table 1). Germination correlated significantly with all five strigolactones in IAC 165 (Fig. 3 A-E). Regression analysis showed that for IAC 165, the three new strigolactones showed the highest contribution to the explanation of variation in

germination (Table 3). For TN 1, however, statistical analysis showed the reverse; orobanchol and 2'-epi-5-deoxystrigol show the highest contribution to the explanation of the variation in germination (Table 3). This could be caused by the fact that there was such a large difference in strigolactone production between the cultivars (Fig. 2 A-J). The concentrations of orobanchol, 2'-epi-5-deoxystrigol and the new strigolactones in most of the TN 1 samples were so close to the detection level (1.5 pmol/mL at S/N >=10) that their quantification is less accurate than in IAC 165, which makes statistical analysis less reliable. Also, there was a strong correlation between the concentrations of the strigolactones (R^2= 0.99 for orobanchol and 2'-epi-5-deoxystrigol; around 0.90 between orobanchol or 2'-epi-5-deoxystrigol and the new strigolactones; data not shown). This makes it difficult to use statistical analysis to discriminate between differences in biological activities of the individual strigolactones. Nevertheless, germination bioassays on fractions of rice root exudates and with standards of orobanchol and 2'-epi-5-deoxystrigol suggest that *Striga* is less sensitive to the latter two strigolactones than to the new strigolactones (unpublished data). For attachment, the conclusions of the regression analysis partially match those for germination in confirming that the new strigolactones significantly explain the variation in attachment in IAC 165. In TN 1, this is now partially confirmed, as new strigolactone 3 was also significantly contributing (Table 3).

Despite the fact that there is such a close correlation between strigolactone peak area in the exudates and germination/attachment, there must be additional parameters that influence germination and possibly also attachment, since the *c.* 100-fold difference in strigolactone concentration between the two varieties is not reflected in a 100-fold difference in germination/attachment. Possible explanations for this discrepancy may be the presence of inhibitors in the exudates of IAC 165 and/or the presence of additional germination inducing compounds in the exudates of TN 1. Indeed, in a study on Nipponbare, fractionation of the root exudates on HPLC suggests that there were additional, possibly strigolactone-like, compounds present in rice root exudates that can induce germination (unpublished data). The involvement of other mechanisms that also affect germination, in addition to the strigolactone concentration, is further confirmed by our study on the NERICA (NEw RICe for Africa rice varieties (Jones et al. 1997a; Dingkuhn et al. 1998). For these varieties, we have found that strigolactones explain most of the variation in *Striga* germination (unpublished data), but variation in attachment and emergence are also explained by a second post-germination resistance factor. Variation in rice for post-attachment resistance has been reported to occur (Gurney et al. 2006; Scholes et al. 2007).

Although it has been shown in several studies that P limitation results in higher strigolactone exudation and germination of parasitic plant seeds (Yoneyama et al. 2007a, b; Lopez-Raez et al. 2008), our study shows for the first time that this increased *in vitro* germination also results in a higher *Striga* infection, as seen in our pot experiment (Table 1). Our results are in line with previous work suggesting that *Striga* spp. infection can be decreased by adding N and P (Farina et al. 1985;

Raju et al. 1990; Ayongwa et al. 2006), but for the first time shows that these effects in the field are likely, at least in part, to be due to a decrease in strigolactone exudation. In our study, the effect of N was less pronounced than the effect of P. Inhibitory effects of nitrogen (especially ammonium) on germination stimulant activity was reported in the past (Yoneyama et al. 2001) **.** However, additional effects of N on *Striga* spp. infection, not mediated through decreased strigolactone production, but due to increasing nitrogen content in the host root or asparagine formation that have been suggested and cannot be excluded (Ayongwa et al. 2006; Simier et al. 2006). The combined depletion of both N and P also gave a lower strigolactone secretion than in the case of just P deficiency (Fig. 2). Too much stress due to the depletion of both N as well as P and the resulting low plant vigour, probably explains the low secretion of strigolactones in this treatment.

As discussed above, strigolactone exudation and *Striga* infection were quite different for the two rice cultivars. IAC 165 exuded about 100-fold more of 2'-epi-5-deoxystrigol, orobanchol and the new strigolactones than cv TN 1 (Fig. 2 A-J) and this is probably causing the higher *Striga* germination and attachment in IAC 165 (Table 1). This suggests the existence of large genetic variation for strigolactone production among rice cultivars. Indeed, in more extensive screening, we confirmed that up to about 500-fold differences exist in the amounts of strigolactones exuded by rice germplasm (unpublished data). Considering the strong correlation between amounts of strigolactone and *Striga* germination/attachment found in the present study, this large genetic variation could be a good basis for breeding against strigolactones and hence for improved *Striga* resistance.

The present results report the potential for using fertilizers as a strategy to suppress *Striga* infection by lowering strigolactone exudation. With the knowledge now available, fertilizer application strategies could be optimized to maintain levels of N and P, such that reduce strigolactone exudation. This could lead to new strategies with optimal *Striga* control and minimal use of scarce fertilizer resources.

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Chapter 6

Striga hermonthica parasitism in maize in response to N and P fertilizers

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Jamil et al. (2011) Field Crops Research (Re-submitted)

Abstract

Parasitism by the parasitic weed, *Striga hermonthica* (*Striga*), constitutes a major biological constraint to maize production in sub-Sahara Africa. Nutrient deficiency is known to aggravate *Striga* infestation and in a number of plant species it was recently shown that this may be due to increased secretion of *Striga* germination stimulants into the soil. The present study was designed to observe the connection between soil fertility, secretion of germination stimulants and *Striga* infection in maize under greenhouse and field conditions. The experiments were conducted during two successive cropping seasons (2008 and 2009). The greenhouse study showed that maize secretes a number of so far unidentified strigolactones that induce *Striga* seed germination and the amount of these strigolactones increases upon N and P deficiency. The increased secretion of germination stimulants under N and P deficiency resulted in increased *Striga* infection in pot experiments. The on-station and on-farm field trials in Western Kenya also showed reduction in *Striga* infestation with the application of mineral nutrients but the results were less consistent than in the greenhouse. Increasing levels of N showed a fair reduction of *Striga* in the field especially during the first year, whereas P application did not have much effect in contrast to the greenhouse study where both N and P clearly reduced *Striga* infection. The likely explanation for this discrepancy is that availability of mineral nutrients under field conditions is less predictable than under greenhouse conditions, due to a number of factors such as soil texture and structure, pH, salinity, drought, leaching and runoff. Hence, further studies are needed on the importance of these factors before a fertilizer application strategy can be formulated to improve control of *Striga* in maize in the field.

Keywords: Strigolactones, Witchweed, Nitrogen, Phosphorus, Zea mays, parasitic weeds, sub-Saharan Africa, Kenya

Introduction

Maize (*Zea mays* L.) is an important cash crop and staple food for many countries in sub-Sahara Africa. Due to its diverse use as food (thick porridge, snack food, pastes, grits, roasted or boiled cobs, and beer), animal feed and industrial raw material, maize use is increasing day by day in African countries and at present the average per capita consumption is over 100 kg per year (Pingali, 2001). Factors like high productivity and low input or labour requirements make maize an attractive crop for farmers in Africa and it is the most widely grown cereal crop in the region. At present maize has the largest acreage of all field crops in Africa, and during 2008 about 29 million hectares was under maize cultivation which accounted for 18% of the 161 million hectares of maize grown globally in that year (FAO, 2009). However, the total maize production during 2008 in African countries was about 55 million tons, which is only 7% of the 826 million tons of maize that was produced globally. The average yield of maize in most African countries is about 1.6 t ha^{-1} (FAO, 2009), which is much lower than the world average yield of 5 t ha^{-1}.

The relatively low maize production in Africa is due to a number of abiotic and biotic constraints. The major abiotic constraints include drought and declining soil fertility (Vanlauwe et al., 2006) while the biotic constraints comprise maize diseases, stem borers and *Striga* infestation (Kanampiu et al. 2003; Khan et al. 2006). *Striga* is considered to be one of the most serious constraints to maize productivity in African agriculture (Gethi et al. 2005). It is a root parasitic weed that damages cereal crops by draining off water and nutrients, impairing photosynthesis and causing a phytotoxic effect within days of attachment to its hosts (Gurney et al. 2006). *Striga* is responsible for an annual loss in cereals worth US$ 7 billion in sub-Saharan Africa (Gethi et al. 2005).

The Lake Victoria Basin in Kenya, a representative site for the present study, is considered to be severely infected by *Striga* especially in maize fields. *Striga* has infested about 0.24 million hectares or about 15% of the arable land in the Lake Victoria Basin alone, causing yield losses between 10% to total crop failure (Smaling et al. 1991) or monetary losses of US$ 41 million (Kanampiu et al. 2003). Grain yields under farmer's field conditions in the Lake Victoria Basin (0.5-1.0 t ha^{-1}) were observed to be less than 70% of the potential yield of 4–5 t ha^{-1} (Tittonell et al. 2005). Nitrogen (N) and phosphorus (P) have been identified as the main deficient nutrients (Vanlauwe et al. 2006) and *Striga* infestation has been found to be closely linked with this deficiency (Vanlauwe et al. 2008). Many studies have reported a decrease of *Striga* infestation with the application of N and P nutrients (Gacheru and Rao 2001; Adagba et al. 2002).

Striga is very prolific, with an individual *Striga* plant producing thousands of tiny dust-like seeds that can remain viable in the soil for 20 years (Winch 2007). *Striga* seed germination is dependent on signalling molecules known as strigolactones. Under mineral nutrient deficiency, host plants secret these strigolactones into the rhizosphere to stimulate the symbiotic relationship with

arbuscular mycorrhizal (AM) fungi that can help the plant to overcome nutrient deficiency (Bouwmeester et al. 2003; 2007). However, parasitic plants also use these signalling molecules to detect the presence of a suitable host. The strigolactones will induce seed germination in *Striga* after which the parasite will attach to the roots of the host and starts to parasitize it. Although the relationship between soil fertility and the *Striga* problem is since long known, a more detailed knowledge on the causal mechanism and the possible relationship with changes in strigolactone production in response to fertility status would be valuable for the optimization of *Striga* control in cereals. The relationship between strigolactones and *Striga* infection and the effect of nitrogen and phosphorus on this has already been demonstrated in rice (Jamil et al., 2011). Rice releases more strigolactones upon lower availability of N and P hence inducing more *Striga* germination which results in higher *Striga* infection. The dramatic effect of N and P starvation on strigolactone production has also been shown in other plant species such as red clover and tomato (Yoneyama et al. 2007a; 2007b; Lopez-Raez et al. 2008). All this suggests that fertilizer application could play a vital role in reducing germination stimulant production and hence, possibly, *Striga* emergence in the field.

The present study was therefore designed with the aim to investigate the effect of fertility and P levels on strigolactone production and consequently on *Striga* infestation in maize both under greenhouse and field conditions. The aim of the study was to provide the scientific basis required to develop *Striga* control strategies in maize using fertilizer application.

Materials and methods

Experimental Sites

The greenhouse study was conducted in Wageningen, the Netherlands, while the field trials were carried out at two different sites in two seasons (2008 and 2009) in Western Kenya. One field study was conducted on-station under artificial *Striga* infestation at the Kenya Agricultural Research Institute (KARI) - International Maize and Wheat Improvement Centre (CIMMYT) *Striga* research facility at Kibos (latitude 0° 4' 0S and 34° 49' 0E, altitude 329 m a.s.l.). The second field study was performed under natural *Striga* infestation in a farmer's field at Baridi, Kenya (about 20 km from the KARI-CIMMYT Kibos facility), a hot spot for *Striga* infestation. The experimental field plots were laid on soils with sandy loam texture. The soil pH levels and fertility status before sowing and after harvesting of the experiments are given in Table 1. The initial status of N and P nutrients of the washed river sand used in the greenhouse experiments in the Netherlands was maintained at a similar level as found in the soil analysis of Kenya.

Seeds, sowing seasons and growth conditions

The variety used in this study was a commercially available high-yielding maize (*Zea mays* L.) hybrid PHB-3253, purchased locally in Kenya. The same cultivar was also used in Netherlands. *Striga* seeds used in the field trial were collected from a maize field (Baridi farm, Kenya) and *Striga* seeds used in

Table 1 Soil chemical properties of experimental fields in Western Kenya before and after maize planting.

	Kibos, Kenya					Farmer's field, Baridi, Kenya				
	pH	Total N	Available N	Total P	Available P	pH	Total N	Available N	Total P	Available P
		(%)	(ppm)	(%)	(ppm)		(%)	(ppm)	(%)	(ppm)
Before trial		-	6.6	-	49.4	-	-	9.3	-	6.8
After trial (Urea : Triple Super Phosphate)										
0:0	5.4	0.05	0.9	0.03	14.8	4.8	0.08	2.9	0.05	1.5
30:0	5.4	0.04	10.5	0.04	13.0	4.8	0.08	4.4	0.04	9.3
60:0	5.3	0.05	2.0	0.03	13.3	4.9	0.08	3.0	0.04	1.6
90:0	5.4	0.05	3.3	0.03	13.7	4.8	0.06	4.1	0.03	1.5
0:25	5.5	0.05	1.1	0.04	18.4	4.7	0.09	2.6	0.04	7.1
0:50	5.1	0.04	0.9	0.03	19.3	5.0	0.09	36.4	0.04	4.3
0:75	5.4	0.03	0.8	0.03	13.8	4.9	0.09	6.31	0.04	4.3
30:25	5.5	0.05	53.9	0.03	14.6	4.9	0.08	2.9	0.04	2.5
60:50	5.2	0.05	19.6	0.01	14.5	5.0	0.08	32.0	0.05	1.9
90:75	5.0	0.05	7.4	0.03	23.4	4.9	0.10	2.7	0.05	3.5

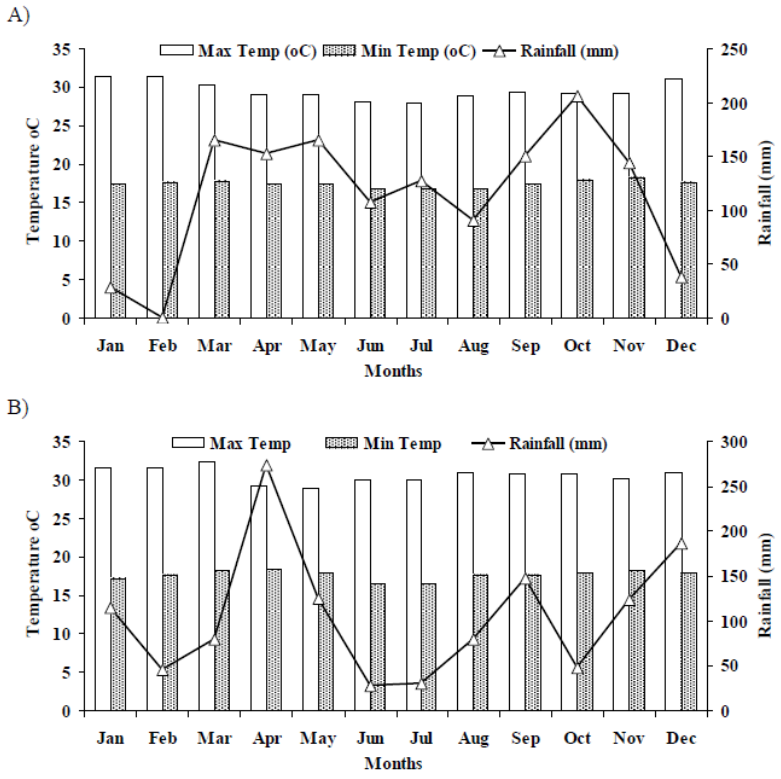

Fig. 1. Meteorological data of field experimental site (Kisumu) during 2008 (A) and 2009 (B) obtained from Kenya Meteorological Department. The bars represent average maximum and minimum temperature (in ^{o}C) while the line diagram represents monthly average rainfall (in mm).

the pot trial were collected from a sorghum field (Wad Medani, Sudan). The viability of *Striga* seeds was in the range of 60-70%. The field experiments were conducted during two cropping seasons, 2008 and 2009. The greenhouse study was completed under controlled conditions (28°C day for 10 h / 25°C night for 14 h; 65% relative humidity throughout) in Wageningen (2008 and 2009). The climatic conditions for the field studies in Kenya are given in Fig. 1. The average mean temperature of the study sites in Kenya was 30^{o}C during the cropping season. The experimental details for the studies at Wageningen, the Netherlands and Kenya (KARI-CIMMYT, Kibos and farmer field Baridi) are shown in Table 2.

Greenhouse experiments at Wageningen University, the Netherlands

Exudate collection and germination bioassays

To assess whether the maize germination stimulants are strigolactones, maize plants were exposed to phosphate starvation and/or treated with the strigolactone biosynthesis inhibitor fluridone after which root exudates were collected for germination bioassays. Maize seeds were germinated on moist rockwool at 28^{o}C for 48 h. The germinated seeds (6 seeds per pot in 4 replicates) were planted in 1.5 L pots filled with 1 L sand which were placed in the greenhouse. The plants were allowed to grow for four weeks, during which half-strength modified Hoagland's nutrient solution with normal P was applied (250 mL per pot at 48 h intervals). In the 5th week after planting, P was removed from all pots by rinsing the pots with 3 L of nutrient solution without P. Hereto, P deficient nutrient solution was applied on top of each pot and allowed to drain from the bottom of the pot. After washing and draining, nutrient solution was applied with 100%P, 10% P and 10%P+fluridone (0.01 µM). These treatments were continued for one week by watering with these solutions when necessary. Seven days after the treatments were started, the pots were again washed with 2.0 L nutrient solution of the corresponding treatments to remove any accumulated exudates. After 48 h, root exudates were collected in plastic bottles by draining the pots with 1 L of nutrient solution of the respective treatments. The collected root exudates were passed through a SPE column (Grace Pure, SPE C18-Fast, 500 mg/3 mL, Alltech, the Netherlands). The strigolactones were eluted with 6 mL of 100% acetone from each column. These samples were used for *Striga* germination bioassays as described by Jamil et al. (2011).

Subsequently new plants were grown under various levels of N and P for exudate collection as well as assessment of *Striga* infection by following the procedure as mentioned above. The corresponding levels of mineral nutrients were maintained in each pot by applying nutrient solution with the respective dose of N and P for one week. Seven days after the treatments were started, the pots were again washed with 2.0 L nutrient solution of the corresponding mineral nutrient composition to remove any accumulated exudates. After 48 h, root exudates were collected in plastic

bottles by draining the pots with 1 L of nutrient solution with the respective N and P nutrient composition and strigolactones were eluted with 6 mL of 100% acetone from C18 fast column as described above. These samples were used for LC-MS/MS analysis and *Striga* germination bioassays.

Strigolactone analysis

Strigolactone analysis was performed using a Waters Micromass Quattro Premier XE tandem mass spectrometer equipped with an electrospray ionization source and coupled to an Acquity UPLC system (Waters) as described by Lopez-Raez et al. (2008). MRM transitions (channels) for the putative maize strigolactone 1 (SL1) eluting at 7.9 min were *m/z* 349 > 217 at a collision energy (CE) of 20 eV and a cone voltage of 20eV, 331 > 217 at CE of 15 eV and a cone voltage of 15eV, 331 > 175 at CE of 18 eV and a cone voltage of 15eV 331 > 97 at CE of 20 eV and a cone voltage of 20eV (named SL1). The MRM channels *m/z* 377 > 97 at a collision energy of 20eV, 377 > 345 at 15eV and 345 > 97 at 20 eV and cone voltage of 15eV were used for the putative strigolactone 2 (SL2) eluting at 10.8 min. The MRM channels *m/z* 331>97 and 377>345, that gave the highest peak intensities, were used for the quantification of SL1 and SL2. Data acquisition and analysis were performed using MassLynx 4.1 (Quanlynx) software (Waters).

Striga infection

In a separate experiment, *Striga* attachment and emergence under different levels of soil fertility were studied in a completely randomized design experiment with four replicates under greenhouse conditions. About 25 mg (about 4000 seeds) *Striga* seeds were weighed for each treatment and mixed thoroughly in 500 mL washed river sand. Plastic pots of 1.5 L volume were taken and perforated plastic sheet placed on the bottom of the pot. About 200 mL sand without *Striga* seeds was placed in the bottom of the pot. Then 500 mL sand with *Striga* seed were added followed by another 100 mL sand without *Striga* seeds. Two germinated maize seeds per pot were planted. The seedlings were thinned to 1 plant per pot 7 days after planting. The seedlings were allowed to grow in the greenhouse. Each pot was assigned different levels of N and P by half strength modified Hoaglands nutrient solution, as described above. *Striga* emergence as well as attachment was assessed 8 weeks after planting after washing of the sand from the roots (rinsing) of the maize plants.

Field Study in Kenya

Two field studies were carried out in Kenya. In the first study at CIMMYT-KARI, Kibos, the soil was infested artificially with *Striga* seeds. *Striga* inoculum was prepared by mixing 5 kg of fine sand and 5 g of *Striga* seeds. One table spoon full of *Striga* inoculum was applied in each planting hill. The maize seeds were placed on top of the inoculums and covered properly. The experiment was laid out in a randomized complete block design with either four (during 2008) or three (during 2009)

replicates (Table 2). The maize crop was sown on 1 May in 2008 and 26 March in 2009. Various levels of N and P (0, 33%, 66%, and 100%) were applied in the form of Urea (46% N) and Triple Super Phosphate (45% P_2O_5). The *Striga* emergence, flowering and mature plants were counted weekly in two inner rows. The final emergence was counted at 12 weeks after planting (WAP). After final counting of flowering and mature plants at 15 WAP, the *Striga* was harvested and dried to determine its dry biomass. The crop was harvested on 10 September in 2008 and 7 August in 2009, after which the number of maize ears and grain yield were measured in the two inner rows.

In the second study the response of *Striga* to various levels of N and P was assessed under natural *Striga* infestation at Baridi's farmer field near Kisumu, Kenya. The experiment was conducted in a randomized complete block design with four (2008) or three (2009) replicates (Table 2). The maize crop was sown on 1 May in 2008 and 10 April in 2009. The sowing dates differed between the years because under the farmer's field conditions the trials were depending on rain. The rains in 2008 started later than in 2009. In Kibos, the on-station trials could be planted without waiting for rain because of availability of irrigation facilities there. Two different plant spacing were used (0.25 and 0.5 m) but these resulted in the same plant density as at 0.50 m plant spacing 2 seeds per hill were planted and at plant spacing of 0.25 m 1 seed per hill. We did not observe any effect of this different plant spacing on *Striga* emergence especially by 12 WAS. Generally farmers plant at a spacing of 0.75 X 0.25 m, 1 seed per hill or 0.75 X 0.50 m, 2 seeds per hill. Urea and TSP were applied according to the treatment (Table 2) in each hill. *Striga* emergence was scored every 2 weeks starting from week 6 until 12 WAP in two inner rows.

Table 2 Experimental details at Wageningen University, Netherlands and Western Kenya.

Parameter	Wageningen Univ. Netherlands		Kibos, Kenya		Farmer's field Baridi,	
	1st year (2008)	2nd year (2009)	1st year (2008)	2nd year (2009)	1st year (2008)	2nd year (2009)
Replications	4	4	4	3	4	3
Row length	-	-	5 m	4.5 m	5 m	4.5 m
Row width	-	-	0.75 m	0.75 m	0.75 m	0.75 m
Plot size/pot size	1.5 Liter	1.5 Liter	15 m^2	13.5 m^2	15 m^2	13.5 m^2
Plant spacing	Single pot^{-1}	Single pot^{-1}	0.5 m	0.25 m	0.5 m	0.25 m
Sowing date	June 19	May 22	May 1	Mar. 26	May 1	Apr. 10
Harvesting date	Sep. 1	Aug. 4	Sep. 10	Aug. 7	Nov. 9	Oct. 8
Striga infestation	20 mg pot^{-1}	20 mg pot^{-1}	1 table spoon	1 table spoon	Natural infestation	Natural infestation
Area harvested	1.5 L pot	1.5 L pot	7.5 m^2	6.75 m^2	7.5 m^2	6.75 m^2
N-source	NH_4NO_3 + KNO_3	NH_4NO_3 + KNO_3	Urea (46%N)	Urea (46%N)	Urea (46%N)	Urea (46%N)
P-source	K_2HPO_4	K_2HPO_4	TSP (45%P_2O_5)	TSP (45%P_2O_5)	TSP (45%P_2O_5)	TSP (45%P_2O_5)

The flowering and mature plants were also counted biweekly starting from 11 WAP to 15 WAP. After final counting of *Striga* flowering and mature plants, *Striga* was harvested and dried to determine its

dry biomass. The maize crop was harvested on 9 November in 2008 and 8 October in 2009. The *Striga* plants were counted at 12 WAS and sampling was done at 15 WAS. Generally the maize stopped producing new root at 12 WAS and maximum number of *Striga* emergence (of different developmental stages) could be expected at this stage. After 12 WAS, the maize crop started to mature and attached/emerged *Striga* started to decline with maximum flowering and maturity occurring around 15 WAS. Maize biomass and other yield components were measured at the time of harvesting as described above.

Statistical and economic analysis

Data were analysed using GenStat Release 9.2 (PC/Windows XP, VSN international Ltd, UK). Multiple comparisons among treatment means (least significance difference test, LSD at P <0.05) and linear as well as quadratic relationships among various treatment were calculated using Fisher's analysis of variance. The two years field grain yield data from both field sites were used to calculate economic and marginal analyses on the basis of prevailing market prices in Kenya by following the procedure devised by CIMMYT (1988). The gross benefit for each fertilizer treatment was calculated by multiplying the sale price with the maize grain yield. The total costs that vary for each treatment were subtracted from the gross benefits of the corresponding treatment to calculate the net benefit. The marginal cost or marginal net benefit represents the increase in total cost or net benefit, respectively, when comparing one production alternative (treatment) to another. The treatment with less benefit and high cost would be uneconomical and by further calculation, marginal rate of return (MRR) was estimated. Marginal rate of return (MRR) is the ratio of the additional benefit over the additional cost. Marginal rates of return were calculated in a stepwise manner from a lower cost treatment/technology to the next higher cost treatment or technology. This information is useful for making decisions or recommendations for farmers or producers to select alternative technologies.

Results

Greenhouse study in Wageningen

To evaluate whether the African adapted maize variety PHB-3253, used in this study produces germination stimulants and whether these are strigolactones, a preliminary study was conducted to assess the effect of P starvation (which should enhance strigolactone production) and fluridone treatment (will inhibit strigolactone production) of maize on the induction of *Striga* germination by the maize root exudates. Root exudates were collected from maize, grown under 100% P, 10% P or 10% P + 0.01µM fluridone and applied to pre-conditioned *Striga* seeds. The highest germination was induced by exudates of plants grown under P deficiency while exudates of plants grown under sufficient P availability or under P deficiency in combination with fluridone induced much less

germination (Fig. 2), suggesting that maize variety PHB-3253 produces strigolactone germination stimulants.

Upon LC-MS/MS analysis, however, no known strigolactones could be detected. But two new putative strigolactones (SL 1 and SL2) were detected at the retention times 7.9 min (SL1)

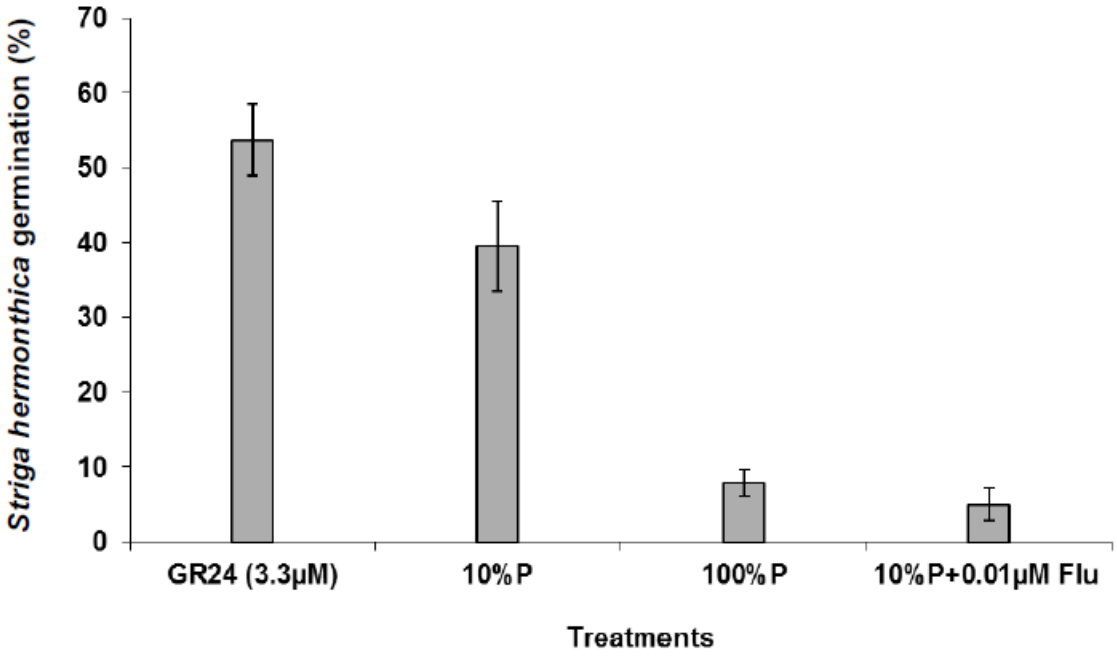

Fig. 2. *Striga hermonthica* germination as induced by root exudates of maize hybrid PHB 3253. The root exudates were collected from plants grown under -P, +P and -P+fluridone and were applied to pre-conditioned *Striga* seeds to assess germination. Bars represent means ± SE (*n*=3).

and 10.8 min (SL2). They showed all the typical characteristics of strigolactones. For example, SL1 was detected at MRM channels *m/z* 349>97 and 331>97 and SL2 at *m/z* 377>97 and 345>97. The detection of a fragment of 97 or the loss of 97 in all these channels shows that the unknown compounds have a D-ring which is characteristic of strigolactones.

After confirming the germination stimulating activity of root exudates of maize plants and the fact that this activity is likely due to the presence of strigolactones, a study was conducted to assess the effect of different N and P levels on production of these unknown strigolactones. For this purpose maize was grown for five weeks in the greenhouse, subjected to different levels of N and P and their root exudates were collected and analysed for the two unknown strigolactones using LC-MS analysis. The production of SL1 and SL2 was highest at a P level of 33% (Fig. 3). Also in combination with N, the 33% treatment gave a high production of the strigolactones. The effect of N alone was less clear but also here the 33% treatment gave the highest production of strigolactones 1 and 2. With an increase of the level of mineral nutrient application the production of the strigolactones decreased in both the N, the P and the NP treatments (Fig. 3). The control treatment (0 N and P) showed a lower production than the 33% treatments, likely because the vigour of the maize plants at 0 N and P was too low.

Striga germination with these root exudates, and *in planta* attachment and emergence were studied under these N and P levels (Fig. 4; Suppl. Table S1). The application of root exudates from plants grown under 33% of N, P and NP induced the highest *Striga* germination while an increasing rate of N, P and NP reduced germination by up to 64% at the highest rates, with a significant linear response to the P and NP levels during both years (Fig. 4; Suppl. Table S1). Similarly the nutrient deficiency (0%) resulted in maximum *Striga* attachment (14 to 17 per plant). The increasing levels of nutrients showed a highly significant and linear negative effect on attachment during the second year and a negative trend during year 1. Also *Striga* emergence and total infection showed a negative trend with increasing mineral nutrient levels, although not always significant. However, emergence showed a significant linear response to N in both years. Supply of both N and P (100%) significantly lowered *Striga* emergence up to 100%.

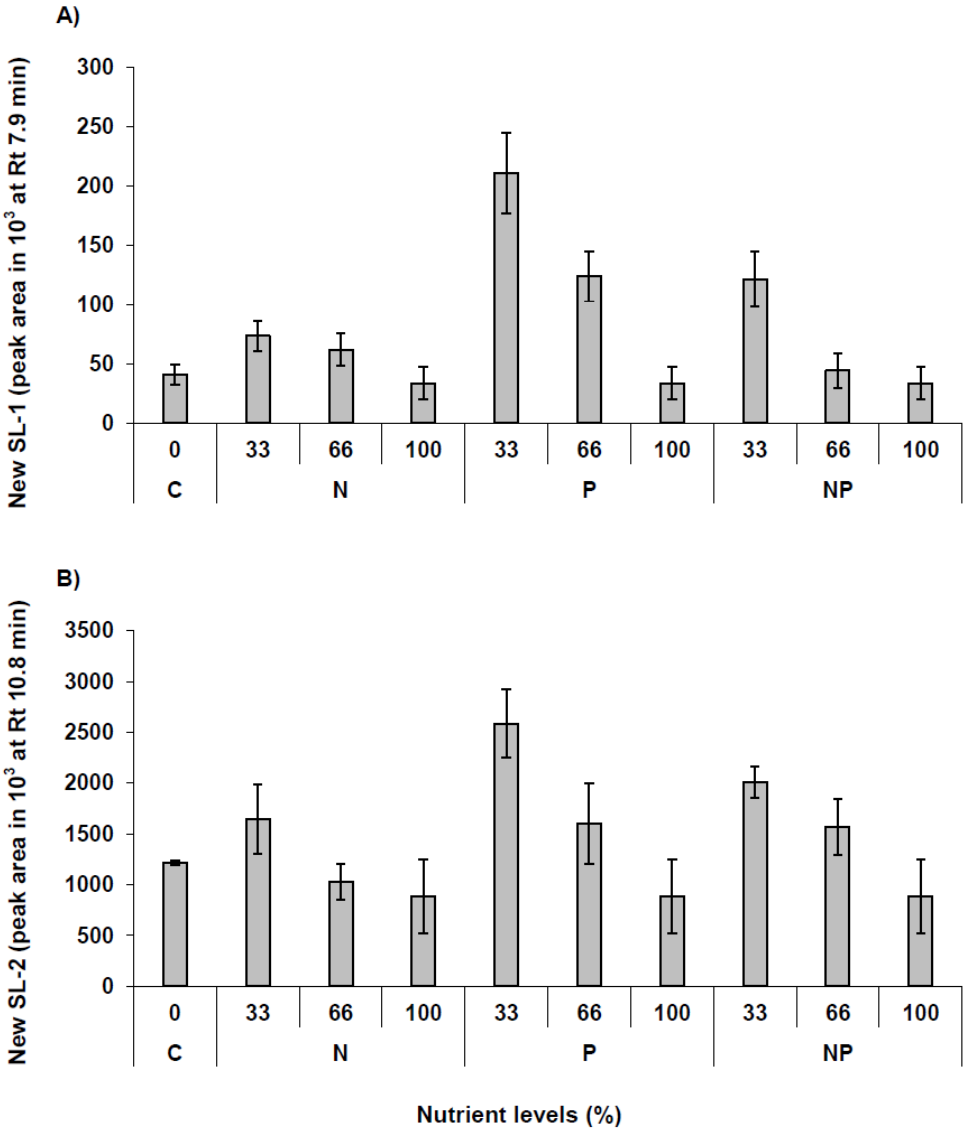

Fig. 3. Peak area (PA) of unknown strigolactones SL-1 (A) and SL-2 (A) in exudates of maize cultivar PHB 3253, under varying levels of nitrogen (N), phosphorus (P) and both N and P (NP). Bars represent means ± SE (*n*=3).

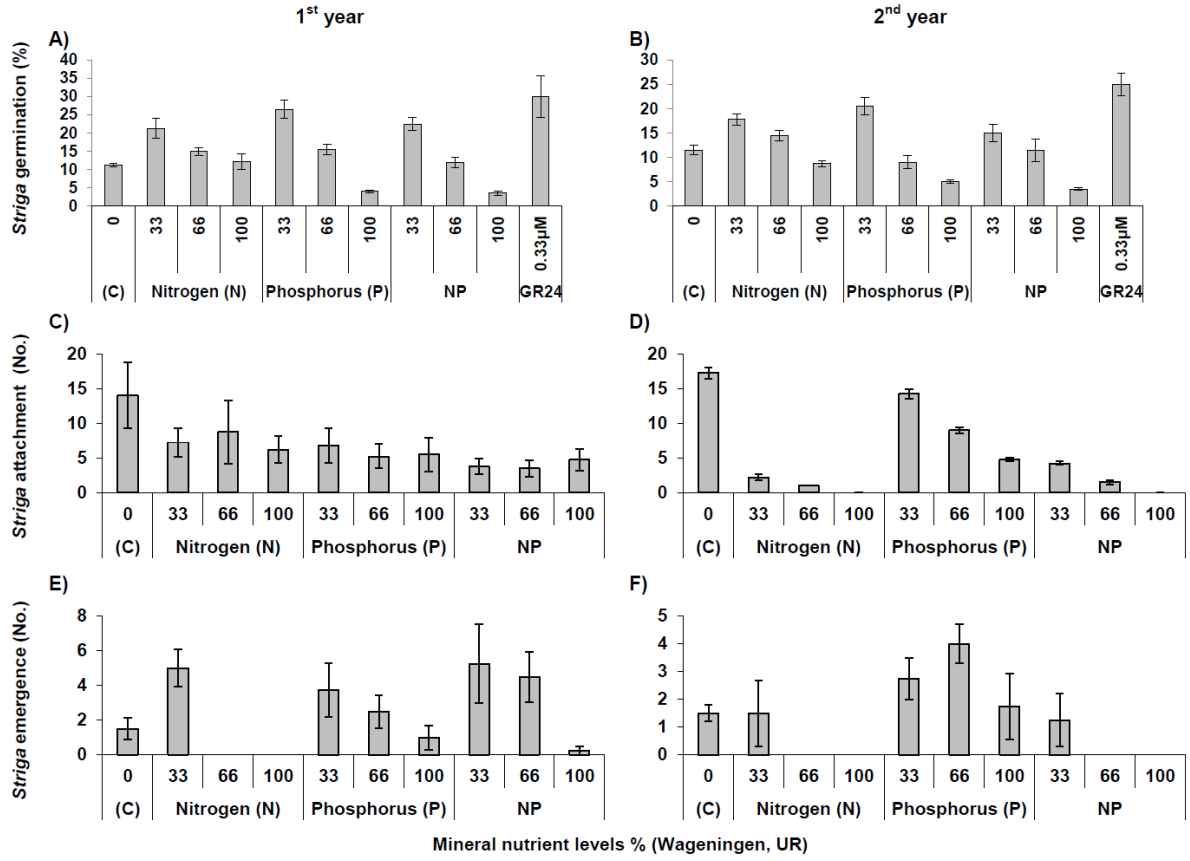

Fig. 4. Effect of different levels of N and P on *Striga* germination (A-B), attachment (C-D) and emergence in a greenhouse experiment in Wageningen. The *Striga* seeds germination was counted after application of maize root exudates collected under various mineral nutrients levels. The attachment and emergence were counted on the roots of maize plant under various levels of N and P. Bars represent means ± SE (*n*=3).

Striga hermonthica emergence and maize yield on-station, Kibos, Kenya

The occurrence of *Striga* at Kibos (Kenya) showed considerable variation between the two years of experiments even though artificial infestation was used (Fig. 5; Suppl. Table S2). About 10-fold more *Striga* emergence was seen in the second year than in the first year. During the first year maximum *Striga* emergence occurred in the fertilizer deficiency treatments (Urea:TSP 0 kg ha^{-1}, Urea 30 kg ha^{-1}, TSP 25 kg ha^{-1} and Ureas+TSP 30+25 kg ha^{-1}) (Fig. 5; Suppl. Table S2). The same holds for the number of *Striga* flowering plants and *Striga* dry biomass (Fig. 5). Increasing levels of Urea and TSP significantly reduced *Striga* emergence, flowering and biomass up to 88-100% compared with the control treatment. In the second year application of both Urea and TSP did not show any significant effect, possibly due to the heavy *Striga* infestation (Fig. 5). Application of Urea and TSP (60:50 or 90:75 kg ha^{-1}) resulted in maximum stalk yield during the first and second year, respectively. In the first year, application of the full dose of Urea and TSP (90:75 kg ha^{-1}) and in the second year a full

dose of only Urea (90 kg ha^{-1}) resulted in maximum grain yield (2306, 2770 kg ha^{-1}) in the on-station field trial in Kibos-Kenya (Suppl. Table S2).

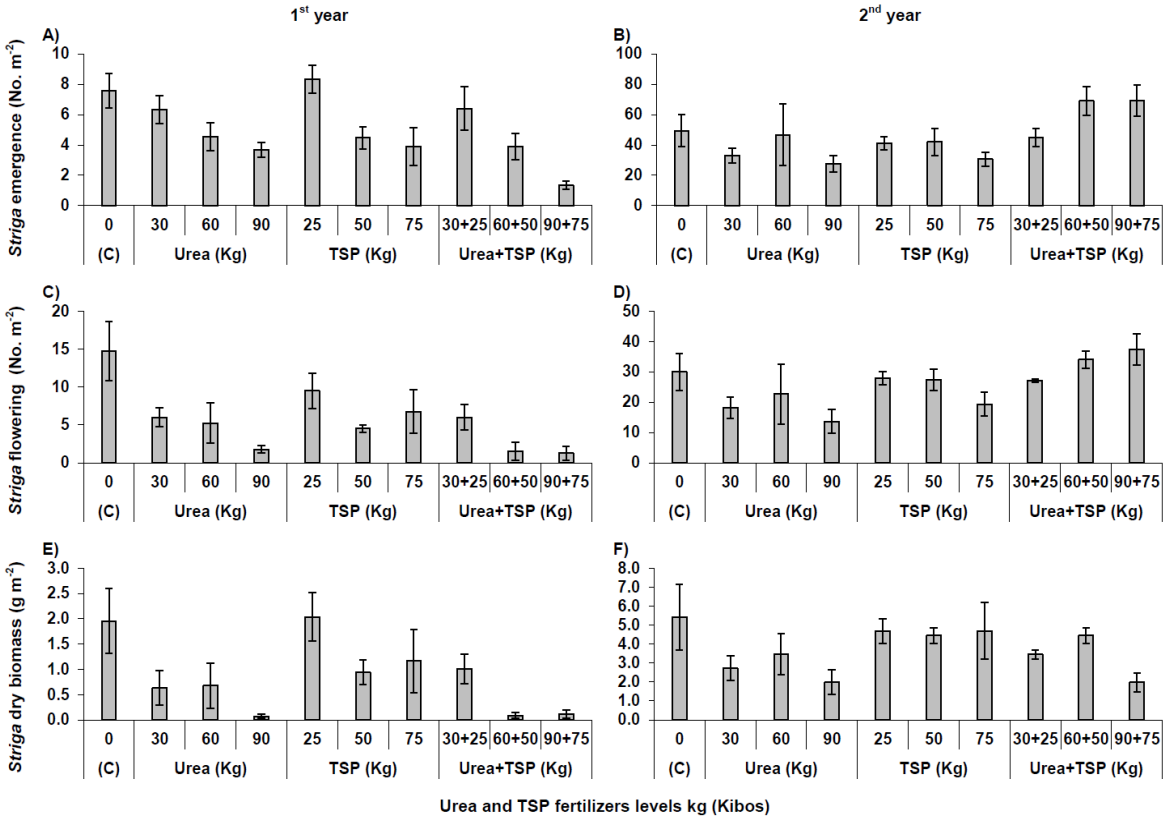

Fig. 5. Effect of different levels of N and P on *Striga* emergence, flowering and dry biomass production at Agri. Res. Station, Kibos, Kenya. The study was conducted under artificial *Striga* infestation during two years. *Striga* emergence was counted at 12 weeks after planting (A-B). *Striga* flowering was counted at 15 weeks after planting (C-D). Dry biomass was determined at harvest (E-F). Bars represent means ± SE (*n*=3).

Striga hermonthica emergence and maize yield on-farm trials, Baridi, Kenya

Under natural *Striga* infestation in a farmer's field in Baridi (Kenya), *Striga* emergence, number of *Striga* flowering plants and dry biomass production did not show statistically significant changes in response to fertilizer treatment although in year 1 there was a negative trend in emergence, flowering and biomass production upon increasing levels of Urea and Urea+TSP (Fig. 6; Suppl. Table S3). Just as in Kibos, there was much more (close to 10-fold) *Striga* infestation during the second year than in the first. Similarly, TSP at 25 kg ha^{-1} (first year) and full dose of both fertilizers at 90:75 kg ha^{-1} (second year) resulted in maximum stalk yield in the farmers field. The grain yield was also maximum during both years with the full levels of Urea and TSP at 90:75 kg ha^{-1} (Suppl. Table S3).

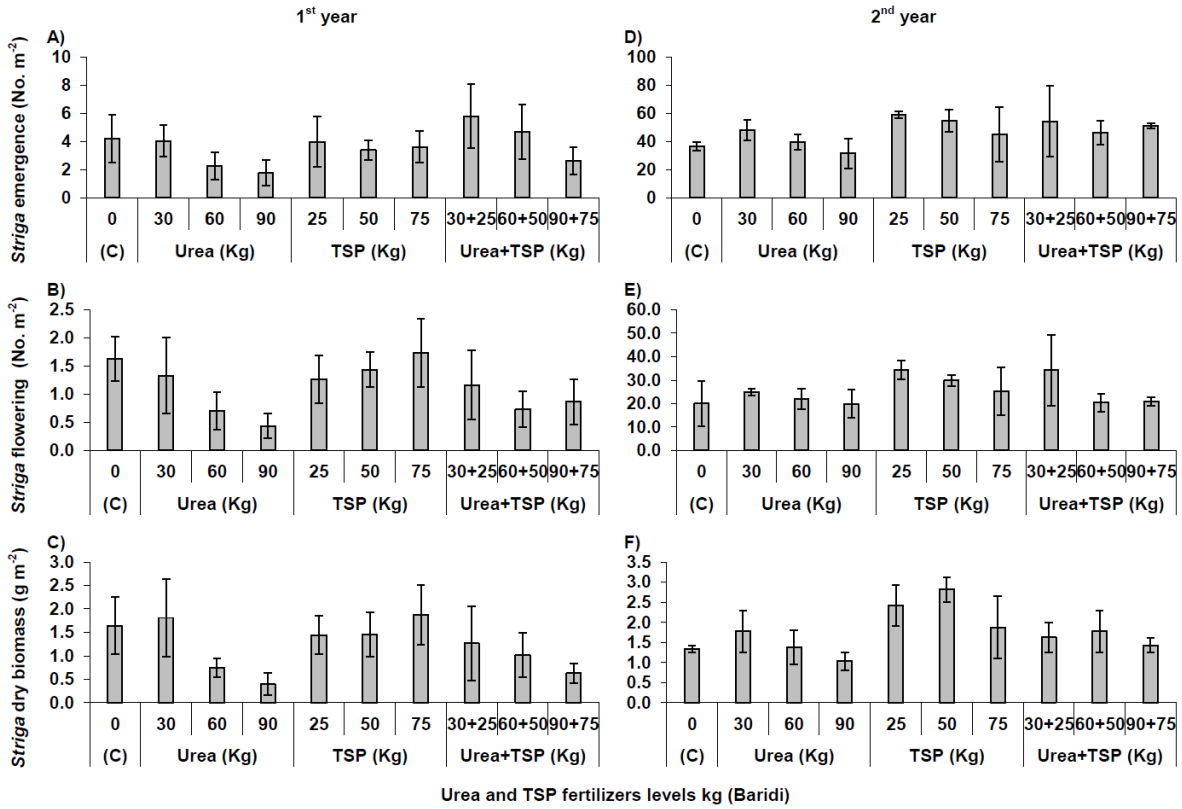

Fig. 6. Effect of different levels of N and P on *Striga* emergence, flowering and dry biomass production in a farmer's field in Baridi, Kenya. The study was conducted under natural *Striga* infestation during two years. Emergence was counted at 12 weeks after planting (A-B). Flowering was counted at 15 weeks after planting (C-D). Dry biomass was determined at harvest (E-F). Bars represent means ± SE (n=3).

Economic and marginal analysis

Economic analysis showed that during the first year at ARS Kibos, the application of 60 kg Urea ha^{-1} resulted in maximum net profit (US$ 333 ha^{-1}) while during the second year 90 kg Urea ha^{-1} gave the highest net profit of US$ 434 ha^{-1} (Suppl. Table S4). In the farmer's field in the first year, application of 25 kg TSP ha^{-1} resulted in the maximum net benefits (US$ 95 ha^{-1}) but in the second year application of Urea:TSP at 90:75 kg ha^{-1} gave the highest net profits of US$ 529 ha^{-1} (Suppl. Table S5). In case of marginal analysis, at ARS Kibos, application of 30 kg Urea ha^{-1} was the best treatment with 1044-1300% marginal rate of return (MRR) for both years (Table 3; Suppl. Table S6). Applications of 60 kg Urea ha^{-1} in both years (950-1490%) and 25 kg TSP ha^{-1} in both years (124-319%) are also seem acceptable based on their MRR%. For the farmer's field 25 kg TSP ha^{-1} with 114% MRR in 2008 and Urea:TSP at 90:75 kg ha^{-1} with 54% MRR in 2009 were the best treatments. The rest of the treatments suffered from either increasing costs that vary or the lower net profits

(Table 3; Suppl. Table S7). So these were uneconomical treatments at the prevailing crop and fertilizers prices.

Table 3 Marginal rate of return calculated based on cost that vary and net benefits under various doses of N and P at ARS, Kibos and farmer's field Baridi Kenya

		Marginal rate of return (%)			
		at ARS, Kibos, Kenya		at Farmer's field Baridi, Kenya	
Fertilizer	Rate	1st year	2nd year	1st year	2nd year
Control	0:0	-	-	-	-
Urea	30:0	1044	1300	-	-
Urea	60:0	950	1490	-	-
Urea	90:0	-	330	-	-
TSP	0:25	124	319	114	-
TSP	0:50	-	-	-	-
TSP	0:75	-	-	-	-
Urea:TSP	30:25		133	-	-
Urea:TSP	60:50	-	-	-	-
Urea:TSP	90:75	-	-	-	54

(For detail see Suppl. Tables S5-S8 and material and method sections)

Discussion

The interaction between the plant's nutritional status and strigolactone (germination stimulant) production, *Striga* germination and AM fungi has been proposed and discussed in several past studies (Bouwmeester et al. 2007; Yoneyama et al. 2007a; 2007b; Lopez-Raez et al. 2008). The struggle by the host plant to improve its nutritional status is the main driving force in this interaction. Under nutrient deficient conditions, plants stimulate AM fungi to engage in symbiosis through enhanced strigolactone signalling. In this symbiosis, the AM fungi provide minerals nutrients, particularly P and N, to the plant in return for host plant-produced carbon. Parasitic plants use this intimate signalling relationship also for host detection, and to engage in a non-symbiotic relationship in which they give nothing in return for host-derived carbon, water and minerals. Indeed, *Striga* is particularly a pest of low fertile soils. Large losses in crop yield (10-100% in maize, 5-95% in sorghum, over 50% in rice) have been reported (Johnson et al. 1997; Kamara et al. 2008) and generally N and P deficiency accentuate the severity of damage to the hosts by this weed (Adagba et al., 2002). Indeed deficiency of particularly these mineral nutrients was found to promote the exudation of strigolactones that induce germination of *Striga* and other root parasitic weeds such as broomrapes (Yoneyama et al. 2007a; 2007b; Lopez-Raez et al. 2008; Jamil et al. 2011).

Although known strigolactones were not detected in the exudate of the maize variety used in our study, the compounds we assume are strigolactones show all the characteristics of strigolactones. The chemical structure of all known strigolactones is quite similar (Zwanenburg et al. 2009). The protonated molecular ions of all strigolactones give (besides fragment ions that are specific for individual strigolactones) also fragment ions common for all strigolactones, such as fragment ion

$C_5H_5O_2$, corresponding to the D-ring with *m/z* 97 (MRM-channels 349>97 and 377>97). The fragment ions *m/z* 345, 331 and 234 correspond to fragments of the ABC-rings of the strigolactone molecules and they are also commonly found for known strigolactones under the same conditions of mass spectrometry (Lopez-Raez et al. 2008; Kohlen et al. 2011; Charnikhova et al. unpublished results). In addition, the response of these compounds to P starvation and the use of carotenoid inhibitors further confirm that they are strigolactones (Fig. 2). The exudation of these two new, unknown strigolactones was highest under the lowest N, P and NP-levels and decreased with increasing fertilizer application (Fig. 3). In general, *Striga* infection under greenhouse conditions followed a similar trend: increasing levels of N, P and NP reduced germination, attachment and emergence (Fig. 4; Suppl. Table S1). However, there are some discrepancies. Application of root exudates collected at 100% P or NP induced the lowest *Striga* germination in both years, while N alone and NP were most effective in reducing *Striga* attachment and emergence. This suggests that N might have a direct effect on *Striga* infection in addition to its (small) inhibitory effect on strigolactone secretion. The increased microbial activity and/or excessive growth and vigour of the host plant have also been mentioned as possible explanations for the inhibitory effect of nitrogen application on *Striga* infestation (Cechin and Press 1993). The excessive accumulation of nitrogen (as NO_3, NO_2 and NH_4) in the host especially in root tissues as a result of nitrogen fertilizer application has been suggested to suppress *Striga* (Ayongwa et al., 2006) either directly possibly due to asparagin formation (Simier et al. 2006) or indirectly due to modification or reduction in germination stimulants (Ayongwa et al. 2006). Nevertheless, in the greenhouse study *Striga* infection to a large extent directly depends upon the amount of strigolactones secreted by the maize host into the rhizosphere. The strong effect of N and P starvation on strigolactone production and *Striga* infection under greenhouse conditions suggests that a reduction in strigolactone production can play an important role in the effect of fertilizer under field conditions. Improvement of soil fertility of low fertile agricultural fields, infested with *Striga,* by application of N and P might reduce strigolactone secretion and result in less *Striga* infection. This mechanism could provide a basis for the development of *Striga* control measures in the field.

However, although increasing rates of mineral nutrients under greenhouse conditions reduced *Striga* infection significantly, under field conditions the reduction was significant only in year 1 in the on-station trial at Kibos (Fig. 5; Suppl. Table S2) while in the farmers field no significant reduction was found (Fig. 6; Suppl. Table S3). A number of factors can be responsible for this discrepancy. The inconsistent results in the field trials might be due to the fact that *Striga* infestation methods, fertilizer formulation and applications methods as well as the soil are not the same as in the green house. Particularly the soil physico-chemical properties are different in the field compared with the greenhouse and can vary between fields which could greatly affect *Striga* infection. Indeed it has already been shown in several publications that soil structure or texture can affect *Striga* seed

germinative ability (Kim et al. 1997). A bad soil texture and structure can cause an imbalance in nutrients in the soil which may affect *Striga* infection. In sandy soil due to poor holding capacity the mineral nutrients can be lost more quickly due to leaching. It may cause host plants to secret more strigolactones and hence induce more *Striga* germination. But excessive moisture in heavy clay soil due to poor drainage might lead to poor germination and attachment of *Striga*. Heavy infestation of *Striga* was reported in sandy loam to loam soil due to favourable conditions for *Striga* (Cardwell and Lane 1995; Tarfa et al. 2001). Sanchez et al. (1997) reported that denitrification, volatilization or leaching loss of N fertilizers and adsorption of P fertilizer on soil particles will affect nutrient availability which may cause large differences between fields in the availability of nutrients (Abunyewa and Padi 2003). The soil analysis of the present study showed that at Kibos there was more P at the beginning of the experiment but also at the end, whereas in Baridi, despite P application, the P level was very low, even at the highest TSP application (Table 1). This variation in P status of soils at both sites might be due to difference in soil pH. Since too low or too high soil pH has been reported to reduce the availability of P due to adsorption on soil particles (Lajtha and Harrison 1995). The lower soil pH of the farmer's field at Baridi than at Kibos (Table 1) may have caused P to adsorb more on soil particles. Indeed, even at the highest TSP rate, the available P at the end of the experiment was low throughout the farmer's field in Baridi (Table 1). This low P availability may have resulted in high strigolactone secretion (as we found under greenhouse conditions) resulting in high *Striga* infection even at 100% TSP. The *Striga* seed density and its viability in the soil could be another factor explaining the discrepancies between field results (Rodenburg et al. 2006). In the second year application of both Urea and TSP did not show any significant effect, possibly due to the heavy *Striga* infestation (Fig. 5; Suppl. Table S2). Indeed, at Kibos, where *Striga* was infested artificially, a more significant effect was seen as compared with the naturally infected farmer's field. The uneven distribution of seeds under farmer's field conditions makes it more difficult to get significant results. In addition, it is likely that at a very high *Striga* seed density, the decrease in *Striga* germination as a result of application of fertilizer does not result in a reduction of *Striga* emergence. Maximum number of *Striga* plants, the host can sustain, is still reached even after a reduction in the number of germinated *Striga* seeds. Finally, another big difference between greenhouse and field is the fact that in the latter, climate is not controlled. The importance of climate is clear from the big difference in *Striga* emergence in the field between the two cropping seasons (Rodenburg et al. 2005). There may be a number of reasons for the very low emergence during the first and very high emergence during the second year. In the first year, the crop was sown about one month later than in the second year (Table 2). High and moderate temperatures increase *Striga* infection. Usually the optimum temperature for germination and attachment of *Striga* in the soil has been reported to be between 30-35^{o}C and any deviation could reduce germination (Dawoud and Sauerborn, 1994). The slight increase of 2^{o}C during May-July in the second year may have favoured *Striga* germination and

attachment. Reduction in *Striga* infection due to lower temperatures has been reported in previous studies (Aflakpui et al. 1998). The amount of rainfall or drought can be another reason for the difference in *Striga* infection between the two cropping seasons. During the first year (Fig. 1), frequent rains occurred after maize planting during June-July (117 mm) and this excessive moisture may have reduced *Striga* viability and germination due to seed decay, leaching of germination stimulants and/or onset of dormancy. A long rainy season and erratic rainfall have been reported to reduce the number of *Striga* seeds in soil that germinate and attach (Oswald and Ransom 2004) and early continuous rainfall and excessive moisture have been reported to reduce *Striga* infection in a number of studies (Gbehounou et al. 1996). However, during the second year early rain in May (124 mm) and subsequent drought in the next two months after maize planting in June-July (25 mm rainfall), may have favoured *Striga* germination and further infection as was also reported by (Ramaiah et al. 1983). The two plant spacing used in the two years (0.25 and 0.50 m). So with one and two seeds per hill, respectively, resulted the same plant density. Therefore, by 12 WAS we would expect to get the same *Striga* emergence per unit area since the maize plant roots should have uniformly covered the area. However, it is possible that with closer spacing maize roots cover a larger area at the initial growth stages leading to more *Striga* emergence and hence could have caused differences in *Striga* emergence levels between the years. Finally, the dormancy of *Striga* seeds is known to be associated with moisture and temperature in the soil (Bebawi et al. 1984; Sun et al. 2007). The sensitivity of *Striga* seeds to germination stimulants has been reported to increase during pre-conditioning (warm stratification or loss of dormancy) and then decrease again due to the induction of secondary dormancy (Matusova et al. 2004). The above mentioned meteorological differences in the two cropping seasons and particularly the late sowing in year 1 may have resulted in more dormant *Striga* seeds at the moment that maize was producing strigolactones and hence to much lower *Striga* infection in that year. The avoidance effect of late sowing on *Striga* infection has been described in previous studies (Gbehounou et al. 2004; Ekeleme et al. 2011).

The acceptance and suitability of any treatment ultimately depends upon its economic returns and the costs involved. Higher net benefits were obtained in most of the nutrient levels as compared to the control during both years of study at Kibos. But at the farmer's field only 100% Urea:TSP in 2009 showed maximum net returns. Thirty kg Urea ha^{-1} at Kibos (at US $ 30 ha^{-1}) and 90:75 kg Urea:TSP ha^{-1} (at US $ 151 ha^{-1}) at farmers field showed the maximum marginal rate of return. De Groot (2007) also showed increase in maize grain yield and MRR% with the use of fertilizer in a *Striga* infested maize field trial.

Conclusion

The outcomes of the present study demonstrate that fertilizer application in maize might reduce *Striga* infection, if the field conditions are suitable, and that this reduction is at least partly due to reduced

secretion of germination stimulants into the rhizosphere. However, there is a discrepancy between the clear effects in the greenhouse and the less consistent results in the field. This shows that more knowledge of the effect of soil physico-chemical properties, *Striga* seed density and climate on the availability of minerals and the biology of *Striga* is required before a fertilizer strategy can be formulated to reduce the *Striga* problem in maize and other cereals in farmer's fields in the African continent.

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Suppl. Table S1 *Striga hermonthica* germination, attachment, emergence and infection under varying levels of nitrogen (N), phosphorus (P) or NP at greenhouse conditions in Wageningen.

Parameter	*Striga* germination		*Striga* attachment		*Striga* emergence		*Striga* infection	
	(%)	(%)	(No. plant^{-1})	(No. plant^{-1})	(No. plant^{-1})	(No. plant^{-1})	(No. plant^{-1})	(No. plant^{-1})
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
NH_4NO_3+KNO_3: K_2HPO_4								
0:0	11(±0.5)[a]	12(±1.0)	14(±4.7)	17(±0.9)	2(±0.6)	2(±0.9)	16(±5)	19(±0.8)
33:0	21(±2.7)	18(±1.1)	7(±2.0)	2(±0.5)	5(±1.1)	2(±0.5)	12(±3)	4(±0.9)
66:0	15(±1.1)	15(±1.0)	9(±4.5)	1(±0)	0(±0)	0(±0)	9(±5)	1(±0)
100:0	12(±2.1)	9(±0.6)	6(±1.9)	0(±0)	0(±0)	0(±0)	6(±2)	0(±0)
0:33	27(±2.5)	21(±1.8)	7(±2.5)	14(±0.6)	4(±1.5)	3(±0.6)	11(±2)	17(±1.1)
0:66	16(±1.3)	9(±1.4)	5(±1.8)	9(±0.4)	3(±1.0)	4(±0.7)	8(±1)	13(±0.4)
0:100	4(±0.4)	5(±0.4)	6(±2.4)	5(±0.3)	1(±.0.7)	2(±1.2)	7(±2)	7(±1.2)
33:33	23(±1.8)	15(±1.8)	4(±1.1)	4(±0.3)	5(±2.3)	1(±0.9)	9(±3)	6(±1.2)
66:66	12(±1.4)	12(±2.3)	4(±1.2)	2(±0.3)	5(±0.1)	0(±0)	8(±1)	2(±0.3)
100:100	4(±0.6)	4(±0.3)	5(±1.5)	0(±0)	0(±0)	0(±0)	5(±2)	0(±0)
P	<0.001	<0.001	NS	<0.001	<0.005	<0.003	NS	<0.001
LSD (5%)	1.6	3.9	-	1.2	3.2	2.0	-	2.0

[a.]Means *n*=4 (±SE); [*]*P* <0.05; [**]*P* <0.01; [***]*P* <0.001; [LSD]Least significant differences of means at *P* = 0.05 by ANOVA test

Suppl. Table S2 *Striga hermonthica* emergence and dry biomass, maize stalk and grain yield under varying levels of nitrogen (N), phosphorus (P) or NP (at Kibos, Kenya).

Parameter	*Striga* emergence		*Striga* dry biomass weight		Maize stalk yield		Maize grain yield	
	(No. m^{-2})	(g m^{-2})	(g m^{-2})	(g m^{-2})	(Kg ha^{-1})	(Kg ha^{-1})	(Kg ha^{-1})	(Kg ha^{-1})
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
Urea:TSP (kg)								
0:0	8(±1.1)[a]	49(±10.6)	2.0(±0.6)	5.4(±1.7)	151.7(±30)	123.5(±24)	623(±183)	63(±0)
30:0	6(±0.9)	33(±4.9)	0.6(±0.3)	2.7(±0.7)	126.7(±23)	271.6(±65)	1420(±169)	1196(±347)
60:0	5(±0.9)	47(±20.2)	0.7(±0.4)	3.5(±1.1)	175.3(±39)	321.0(±24)	2080(±145)	2141(±567)
90:0	4(±0.5)	28(±5.4)	0.1(±0.0)	2.0(±0.7)	175.0(±36)	395.1(±49)	1938(±165)	2770(±1056)
0:25	8(±0.9)	41(±4.4)	2.0(±0.5)	4.7(±0.7)	190.3(±32)	148.1(±0)	873(±118)	525(±76)
0:50	4(±0.7)	42(±9.0)	0.9(±0.2)	4.4(±0.4)	155.0(±25)	123.5(±24)	986(±269)	231(±138)
0:75	4(±1.2)	31(±4.7)	1.2(±0.6)	4.7(±1.5)	133.3(±36)	148.1(±0)	771(±198)	336(±91)
30:25	6(±1.4)	45(±5.8)	1.0(±0.3)	3.5(±0.2)	183.3(±22)	246.9(±49)	1179(±258)	1364(±615)
60:50	4(±0.9)	69(±9.3)	0.1(±0.0)	4.4(±0.4)	255.0(±11)	345.7(±49)	2080(±229)	1007(±441)
90:75	1(±0.3)	69(±10.4)	0.1(±0.0)	2.0(±0.5)	163.3(±44)	444.4(±42)	2306(±136)	2036(±736)
P	<0.001	<0.06	0.005	NS	NS	<0.001	<0.001	0.009
LSD (5%)	2.6	28	1.0	-	-	117	506	1410

[a.]Means *n*=3 or 4 (±SE); [*]*P* <0.05; [**]*P* <0.01; [***]*P* <0.001;[LSD]Least significant differences of means at *P* = 0.05 by ANOVA test

Suppl. Table S3 *Striga hermonthica* emergence and dry biomass, maize stalk and grain yield under varying levels of nitrogen (N), phosphorus (P) or NP (at farmer's field Baridi, Kenya).

Parameter	*Striga* emergence		*Striga d*ry biomass weight		Maize stalk yield		Maize grain yield	
	(No. m^{-2}) 1st year	(No. m^{-2}) 2nd year	(g m^{-2}) 1st year	(g m^{-2}) 2nd year	(Kg ha^{-1}) 1st year	(Kg ha^{-1}) 2nd year	(Kg ha^{-1}) 1st year	(Kg ha^{-1}) 2nd year
Urea:TSP (kg)								
0:0	4(±1.7)[a]	37 (±3.1)	1.6 (±0.6)	1.3 (±0.1)	61.3(±11)	518.5(±148)	313(±181)	2300(±894)
30:0	4(±1.1)	48(±7.4)	1.8(±0.8)	1.8(±0.5)	91.7(±23)	395.1(±107)	280(±219)	1280(±546)
60:0	2(±1.0)	40(±5.2)	0.7(±0.2)	1.4(±0.4)	51.3(±20)	345.7(±49)	314(±223)	1218(±435)
90:0	2(±0.9)	32(±10.7)	0.4(±0.2)	1.0(±0.2)	45.3(±7)	567.9(±162)	336(±122)	1956(±427)
0:25	4(±1.8)	59(±2.4)	1.4(±0.4)	2.4(±0.5)	53.3(±16)	321.0(±65)	550(±219)	890(±428)
0:50	3(±0.7)	55(±8.0)	1.5(±0.5)	2.8(±0.3)	30.7(±8)	246.9(±49)	270(±129)	571(±195)
0:75	4(±1.1)	45(±19.4)	1.9(±0.6)	1.9(±0.8)	38.7(±12)	370.4(±42)	257(±239)	1161(±402)
30:25	6(±2.3)	55(±25.1)	1.3(±0.8)	1.6(±0.4)	21.3(±3)	395.1(±65)	191(±50)	1159(±419)
60:50	5(±1.9)	46(±8.5)	1.0(±0.5)	1.8(±0.5)	75.3(±15)	493.8(±130)	508(±236)	1740(±476)
90:75	3(±1.0)	51(±1.7)	0.6(±0.2)	1.4(±0.2)	62.7(±8)	592.6(±85.5)	835(±290)	3211(±427)
P	NS	NS	NS	NS	<0.05	NS	NS	<0.04
LSD (5%)	-	-	-	-	40	-	-	1455

[a.]Means n=3 or 4 (±SE); $^{*}P$ <0.05; $^{**}P$ <0.01; [LSD]Least significant differences of means at P = 0.05 by ANOVA test

Suppl. Table S4 Economic analysis on the basis of maize grain yield (at Kibos, Kenya).

Urea:TSP	**0**	**30:0**	**60:0**	**90:0**	**0:25**	**0:50**	**0:75**	**30:25**	**60:50**	**90:75**	**Remarks**
First year (2008)											
Grain yield	623	1420	2080	1938	873	986	771	1179	2080	2306	Kg ha^{-1}
10% less than actual yield	62	142	208	194	87	99	77	118	208	231	Kg ha^{-1} (at farmer's level)
Adjusted yield	561	1278	1872	1744	785	887	694	1061	1872	2076	Kg ha^{-1}
Gross income	118	268	393	366	165	186	146	223	393	436	Maize price @ $27/90kg bag
Cost of fertilizer	0	26	52	77	19	37	56	44	89	133	Urea $43;TSP $37/50kg bag
Fertilizer application cost	-	4	8	12	2	4	6	6	12	18	$ 4 man day ha^{-1} (Urea 3 man day:TSP 2 man day)
Cost that vary	0	30	60	89	21	41	62	50	101	151	$ ha^{-1}
Net benefit	118	238	333	277	144	145	84	172	292	285	$ ha^{-1}
Second year (2009)											
Grain yield	63	1196	2141	2770	525	231	336	1364	1007	2036	Kg ha^{-1}
10% less than actual yield	6	120	214	277	52	23	34	136	101	204	Kg ha^{-1} (at farmer's level)
Adjusted yield	57	1077	1927	2493	472	208	302	1228	907	1832	Kg ha^{-1}
Gross income	12	226	405	524	99	44	63	258	190	385	Maize price @ $27/90kg bag
Cost of fertilizer	0	26	52	77	19	37	56	44	89	133	Urea $43;TSP$37/50kg bag
Fertilizer application cost	-	4	8	12	2	4	6	6	12	18	$ 3.7 man day ha^{-1} (Urea 3 man day:TSP 2 man day)
Cost that vary	0	30	60	89	21	41	62	50	101	151	$ ha^{-1}
Net benefit	12	196	345	434	79	3	2	208	90	234	$ ha^{-1}

Suppl. Table S5 Economic analysis on the basis of maize grain yield (farmer's field Baridi, Kenya).

Urea:TSP	0	30:0	60:0	90:0	0:25	0:50	0:75	30:25	60:50	90:75	Remarks
First year (2008)											
Grain yield	325	272	325	336	549	261	253	189	501	843	Kg ha^{-1}
Gross income	68	57	68	71	115	52	55	40	105	177	Maize price @ $19/90kg bag
Cost of fertilizer	-	26	52	77	19	37	56	44	89	133	Urea $43;TSP$37/50kg bag
Fertilizer application cost	-	4	8	12	2	4	6	6	12	18	$ 3.7 man day ha^{-1} (Urea 3 man day:TSP 2 man day)
Cost that vary	-	30	60	89	21	41	62	50	101	151	$ ha^{-1}
Net benefit	68	27	9	-	95	14	-	-	5	26	$ ha^{-1}
Second year (2009)											
Grain yield	2390	1244	1264	1956	889	553	1146	1146	1719	3240	Kg ha^{-1}
Gross income	502	261	265	411	187	116	241	241	361	680	Maize price @ $27/90kg bag
Cost of fertilizer	0	26	52	77	19	37	56	44	89	133	Urea $43;TSP$37/50kg bag
Fertilizer application cost	-	4	8	12	2	4	6	6	12	18	$ 3.7 man day ha^{-1} (Urea 3 man day:TSP 2 man day)
Cost that vary	-	30	60	89	21	41	62	50	101	151	$ ha^{-1}
Net benefit	502	232	206	321	166	75	179	190	260	529	$ ha^{-1}

Suppl. Table S6 Marginal Rate of Return based on cost and benefit at research station Kibos, Kenya (2008-2009)

	First year (2008)							Second year (2009)					
	Rate (Kg ha^{-1})	Cost ($ ha^{-1})	N.B[a] ($ ha^{-1})	M. Cost[b] ($ ha^{-1})	M.N.B.[c] ($ ha^{-1})	M.R.R.[d] (%)		Rate (Kg ha^{-1})	Cost ($ ha^{-1})	N.B. ($ ha^{-1})	M. Cost ($ ha^{-1})	M.N.B. ($ ha^{-1})	M.R.R. (%)
Control	0	0	118				Control	0	0	12			
TSP	0:25	21	144	21	26	124	TSP	0:25	21	79	21	67	319
Urea	30:0	30	238	9	94	1044	Urea	30:0	30	196	9	117	1300
TSP	0:50	41	145	11			TSP	0:50	41	3	11		
Urea:TSP	30:25	50	172	9			Urea:TSP	30:25	50	208	9	12	133
Urea	60:0	60	333	10	95	950	Urea	60:0	60	345	10	149	1490
TSP	0:75	62	84	2			TSP	0:75	62	2	2		
Urea	90:0	89	277	27			Urea	90:0	89	434	27	89	330
Urea:TSP	60:50	101	292	12			Urea:TSP	60:50	101	90	12		
Urea:TSP	90:75	151	285	50			Urea:TSP	90:75	151	234	50		

[a] net benefit; [b]marginal cost; [c]marginal net benefit; [d]marginal rate of return

Suppl. Table S7 Marginal Rate of Return based on cost and benefit at farmer's field Baridi, Kenya (2008-2009)

	First year (2008)							Second year (2009)					
	Rate (Kg ha^{-1})	Cost ($ ha^{-1})	N.B[a] ($ ha^{-1})	M. Cost[b] ($ ha^{-1})	M.N.B.[c] ($ ha^{-1})	M.R.R.[d] (%)		Rate (Kg ha^{-1})	Cost ($ ha^{-1})	N.B. ($ ha^{-1})	M. Cost ($ ha^{-1})	M.N.B. ($ ha^{-1})	M.R.R. (%)
Control	0	0	65	-	-	-	Control	0	0	502	-	-	-
TSP	0:25	21	89	21	24	114	TSP	0:25	21	166	21	-	-
Urea	30:0	30	25	9	-	-	Urea	30:0	30	232	9	-	-
TSP	0:50	41	11	11	-	-	TSP	0:50	41	75	11	-	-
Urea:TSP	30:25	50	0	9	-	-	Urea:TSP	30:25	50	190	9	-	-
Urea	60:0	60	5	10	-	-	Urea	60:0	60	206	10	-	-
TSP	0:75	62	0	2	-	-	TSP	0:75	62	179	2	-	-
Urea	90:0	89	0	27	-	-	Urea	90:0	89	321	27	-	-
Urea:TSP	60:50	101	0	12	-	-	Urea:TSP	60:50	101	260	12	-	-
Urea:TSP	90:75	151	18	50	-	-	Urea:TSP	90:75	151	529	50	27	54

[a] net benefit; [b]marginal cost; [c]marginal net benefit; [d]marginal rate of return

Chapter 7

Fertiliser microdosing for *Striga hermonthica* control in sorghum

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Abstract

Striga hermonthica **infection poses a major biological constraint to sorghum production in sub-Saharan Africa and low soil fertility aggravates *Striga* problem to a considerable extent. Under mineral nutrient deficiency, the sorghum host secretes signalling molecules – strigolactones – into the rhizosphere, which induces *Striga* seed germination and subsequent infection of host roots. Fertiliser microdosing is an interesting, cheaper alternative compared with broadcasting. In the current study we analysed the effect of diammonium phosphate (DAP) fertilizer microdosing on production of strigolactones, *Striga* infection, yield potential and economics of three different African sorghum cultivars (CGM-19/1-1, Lina-3, DouaG). Sorghum cultivars produced strigolactones sorgomol and 5-deoxystrigol, albeit in different quantities and ratios. High *Striga* infection and emergence occurred, under greenhouse as well as field conditions, in the unfertilized treatment. DAP microdosing reduced secretion of sorgomol and 5-deoxystrigol, and *Striga* germination (66-70%), emergence (49-73%) and dry biomass (90-96%) under greenhouse conditions. DAP microdosing reduced *Striga* emergence by 40 to 84% and increased sorghum grain yield by 47 to 142% in field. Microdosing with 2 g DAP hill^{-1} proved highly profitable for all varieties compared with control treatment. The present findings show that DAP microdosing reduces secretion of strigolactones into the rhizosphere and *Striga* parasitism both under greenhouse and field conditions. Microdosing of DAP may prove to be an efficient and cost effective option to reduce *Striga* damage in sorghum fields in SSA, particularly in combination with other control options such as varietal resistance, intercropping, organic fertilizer and hand pulling of *Striga* at flowering to achieve integrated *Striga* and soil fertility management.**

Keywords: Strigolactones, *Striga hermonthica*, diammonium phosphate, sorghum

Introduction

Sorghum (*Sorghum bicolor* L.*)* is an important food and feed crop in sub-Saharan Africa (SSA). At present this subsistence crop is grown on about 24 million hectares in Africa which accounts for 60% of the 40 million hectares of sorghum grown globally (FAO 2009). However, total sorghum production in Africa during 2009 was only 22 million tons, which is about 37% of the worldwide production. The average yield of sorghum in most African countries is about 0.9 t ha^{-1} which is substantially lower than the world average yield of 1.4 t ha^{-1}. The current sorghum production per unit area is not sufficient to meet the demand for human consumption, animal feed and fuel or building material requirements of a rapidly growing African population. Drought, bird attacks, insect pests, diseases and parasitic weeds are the major constraints to sorghum productivity in SSA, of which the parasitic weeds *Striga* (Del.) Benth and *S. asiatica* (L.) Kuntze are among the most important biotic constraints (Haussmann et al. 1998; Khan et al. 2006; Khan et al. 2008; Guo et al. 2011). Also degraded soils, nutrient depletion and low soil fertility have been recognised as major factors responsible for low sorghum production in SSA (Palé et al. 2009). The use of fertiliser in sorghum by African farmers is limited as a result of poor accessibility and availability of fertiliser and high fertiliser prices (Bekunda et al. 1997; Bagayoko et al. 2000; Dembele et al. 2000; Bagayoko et al. 2011). It is generally observed that phosphorus (P) and nitrogen (N) are the most limiting nutrients for sorghum production in Africa (Bationo & Mokwunye 1991). Insufficient application of fertilisers limits sorghum productivity by reduced growth and development but also by increased *Striga* infestation. Vice versa, fertiliser application has been shown to suppress *Striga* infection and improves growth and productivity of the host plant (Gacheru & Rao 2001; Oswald & Ransom 2001).

Germination of *Striga* seeds is triggered by the presence of signalling molecules, called strigolactones, in the rhizosphere (Bouwmeester et al. 2003; Bouwmeester et al. 2007), that are secreted by the roots of host and non-host plants (Yoneyama et al. 2007; Lopez-Raez et al. 2008; Jamil et al. 2011a). Increased levels of secretion of strigolactones by roots of host plants has been found under phosphorus (P) and nitrogen (N) deficient conditions (Yoneyama et al. 2007; Lopez-Raez et al. 2008) suggesting that application of fertilisers that contain both N and P, such as NPK and Di-ammonium Phosphate (DAP), can be useful in reducing *Striga* infection indirectly by reducing strigolactones secretion (Jamil et al. 2011a).

The high and increasing cost of mineral fertilisers and low purchasing power of African farmers have necessitated investigating the efficacy of fertiliser application at low to very low levels. The use of very low doses of mineral fertilisers and its placement near the planting hole, a technology termed “microdosing”, has shown to reduce the rate and thus, cost of fertiliser per surface area while still improving crop yields (Tabo et al. 2007). Microdosing has also been reported by farmers to

reduce the negative effect of *Striga* on the host (Aune et al. 2007), but research into the effect of microdosing on *Striga* infection and knowledge about the mechanism involved is lacking.

Therefore, the current study aimed to elucidate the mechanism of microdosing induced reduction in *Striga* infection. To achieve this goal, we used a combination of greenhouse and field experiments with three African sorghum varieties with different reaction to *Striga* and the commercially available fertiliser, diammonium phosphate (DAP) with formulation $(NH_4)_2HPO_4$ (N18%:P46%). DAP fertiliser is widely used and popular among farmers because of its good solubility in water and relatively high percentages of P and N. The three cultivars were tested for strigolactone production and *Striga* infection in bio-assays and pot trials under Dutch greenhouse conditions as well as under Malian field conditions.

Materials and methods

Experimental sites

The greenhouse study was carried out at Wageningen University, the Netherlands. The field trial was conducted at the Samanko research station of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), situated about 20 km South-West of Bamako in Mali (8˚54'W and 12˚54' N; altitude: 329 m).

Seeds, soils and growth conditions

Three sorghum cultivars, CGM-19/1-1, Lina-3, and DouaG were used in the present study. In preliminary field trials with artificial infestation of *Striga*, CGM-19/1-1 showed high emergence levels of *Striga* with low yields and was thus rated susceptible, while Lina-3 showed lower levels of *Striga* emergence and maintained good yield, thus indicating that the variety has some degree of resistance and is tolerant to *Striga*. The variety DouaG appears to be adapted to soils with low phosphorus levels (Rattunde pers. comm), but its reaction to *Striga* is unknown. *Striga* seeds for the pot trial were collected from a sorghum field near Cinzana in Mali (courtesy of Cheickna Diarra). *Striga* seeds used in the germination bioassays were collected from a sorghum field in Wad Medani, Sudan (courtesy of Abdel Gabar Babiker). The greenhouse study was carried out under controlled conditions (28°C day for 10 h / 25°C night for 14 h; 65% relative humidity) in Wageningen University. For strigolactone collection, silver river sand and for the *Striga* infection study in pots, a mixture of soil and sand (50:50) was used. The field experiment was laid out on a sandy loam, ferruginous tropical soil with washout spots and concretions. The soil had a pH of 5.0 (pH-H_2O), organic C content of 0.29%, available phosphorus content of 10.5 (Bray^{-1}; mg P kg^{-1}), total nitrogen content of 204 (mg N kg^{-1}), potassium content of 0.17 (cmol+ kg^{-1}) and a Cation Exchange Capacity (CEC) of 1.8 (cmol+ kg^{-1}). The climate type at the research station is Sudanean, with one rainy season

per year between May and October and an average annual rainfall of 950 mm, calculated over the last 10 years rainfall data. The cropping season of the site runs from the end of June to November, 2010 and the average temperature in this period is 29.1^{o}C. Total rainfall in 2010 was exceptionally high, with 1231 mm in 67 days and in the period between sowing and crop maturity, 1003 mm in 52 days (Suppl. Fig. 1).

Experimental details

The experimental details for the greenhouse study at Wageningen, the Netherlands and the field study at ICRISAT Samanko research station in Mali are shown in Table 1. Both the pot and the field trial had a full factorial, complete randomised design with four replicates, three levels of fertilisation and three sorghum cultivars, leading to a total of 9 treatments. The three levels of fertilisation consisted of application of 0, 2 and 4 gram of DAP fertiliser per plant. In the field trial, the fertiliser was applied in one hole near the plant while in the pot study the same amount of fertiliser was distributed in three small holes around the plant. Further details of the studies conducted in the greenhouse and field were as follows.

Table 1 Experimental detail at Wageningen University, Netherlands and farmer's field Mali

Parameter	Wageningen Univ. Netherlands	Samanko research station, Mali
Replications	4	4
Row length	-	4 m
Row width	-	0.75 m
Plot size/pot size	1.5 L	3 m x 4 m
Plant spacing	Single per pot	0.4 m x 0.75 m
Sowing date	July 19, 2010	June 18, 2010
Harvesting date	August 27, 2010	November 19, 2010
Striga (Seed plant^{-1})	25 mg per pot (4000)	55 mg per hill (9 plants/4 m row) (10000)
Area harvested	1.5 L pot	14 sorghum hills per plot
N and P-source	DAP (18%N:46%P_2O_5)	DAP (18%N:46%P_2O_5)
DAP levels	0, 2, 4 g per plant	0, 2, 4 g per hill

Bioassays and greenhouse pot trial

For strigolactone collection, sorghum seeds were germinated on moist filter paper at 28^{o}C for 48 h. After germination, two seeds per pot were planted in 1.0 L pots filled with 750 mL sand during June, 2010. Seven days after planting, the seedlings were thinned to 1 plant per pot and at this time, DAP fertiliser was applied at 0, 2 and 4 g per plant. Each level of DAP fertiliser was applied in three small holes (5 cm deep) around the plants (6 cm away). The plants were irrigated with half strength Hoagland's nutrient solution without N and P at a dose of 250 mL per pot at 48 h intervals. The plants were allowed to grow for two weeks in a climate chamber at 28°C (10h)/25°C (14 h) photoperiod (supplement with artificial light 400 µM m^{-2} s^{-1}) and 70% relative humidity. In the 3rd week, root

exudates were collected in plastic bottles by draining the pots with 1 L of nutrient solution. The collected root exudates were passed through a C18-Fast column (500 mg 3 mL^{-1}). The strigolactones were eluted with 4 mL of 100% acetone from each column. These samples were purified by using a Silica column (300 mg 3 mL^{-1}). Two mL of the acetone eluent was transferred to a 4 mL glass vial and the acetone evaporated in a vacuum centrifuge. After dissolving the residue in 50 µL ethyl acetate, 4 mL hexane was added. This solution was loaded on a pre-conditioned Silica column (Grace Pure SPE). The strigolactones were eluted using 2 mL solvent mixtures of hexane:ethyle acetate (10:90) using 2 mL solvent mixtures. The solvent mixtures were evaporated again using a vacuum centrifuge and the residue dissolved in 200 µL of 25% acetonitrile. The samples were filtered through Minisart SRP4 0.45 µm filters (Sartorius, Germany) for LC-MS/MS analysis. The remaining 2 mL of the acetone eluent of the C18 column was used for *Striga* germination bioassays as described previously (Jamil et al. 2011a).

Striga emergence was studied in pots in the greenhouse at the same microdosing levels (0, 2 and 4 g per plant) during July, 2010. About 25 mg *Striga* seeds were weighed for each pot and mixed thoroughly with 1 L of the 50:50 sand and soil mixture. Plastic pots of 3.0 L volume were taken and perforated plastic sheet was placed on the bottom of the pot. About 500 mL of soil and sand mixture without *Striga* seeds was placed in the bottom of the pot. Then 1 L of the sand and soil mixture with *Striga* seed was added. On top of this mixture, 200 mL of sand without *Striga* seeds was added. Sorghum seeds were germinated on moist rock wool at 28^{o}C for 2 days. After germination, two seeds per pot were planted. The seedlings were thinned to 1 plant per pot 7 days after planting after which DAP fertiliser was applied at 0, 2 and 4 g per pot. The DAP fertiliser was applied as described above, in three small holes (5 cm deep) around the plants (6 cm away).. The sorghum seedlings were grown in the greenhouse at 28°C day (14 h) and 25°C night (10 h) with relative humidity 70% supplemented with light (400 µM m^{-2} s^{-1}). *Striga* emergence was recorded at two day intervals up to 10 weeks after planting. Then the *Striga* plants were up-rooted, oven dried at 70^{o}C for 72 h and weighed to determine dry biomass.

Field Study at the ICRISAT-Samanko research station in Mali

The field study was carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) at the Samanko research station in Mali. The soil was infested artificially with *Striga* seeds. Sorghum rows of 4 m length were infested with 0.5 g of *Striga* seed on 16 July, 2010. *Striga* seeds were mixed with 500 g of sand and spread out in a furrow of about 10 cm deep and 10 cm wide in a ridge and by subsequently closing the furrow with soil from the sides of the ridge. The sorghum seeds were sown on 18 July 2010 at about 2 cm depth and DAP fertiliser was applied in the same way, at about 5 cm from the planting hole at the same time as sowing. Thinning was done at 10 days after sowing (DAS) and a small number of missing hills were resown at the same time. First weeding

and ridging was done at 25 DAS after which all weeds but *Striga* were weeded by hand every 2 weeks. *Striga* emergence was counted at 120 DAS and *Striga* biomass was determined by accumulating dead and mature *Striga* plants at 90, 120 and 150 DAS. The crop was harvested on 19 November, 2010. The sorghum plants were harvested from 14 hills from the two inner rows and the stalk and grain yield measured after air drying and threshing.

Statistical and economic analysis

Data collected were statistically analysed using GenStat Release 9.2 (PC/Windows XP), VSN international Ltd, UK. Multiple comparisons among treatment means (least significance difference test (LSD) at P <0.05 and linear relationships among various treatments were calculated using Fisher's analysis of variance (ANOVA). Data from *Striga* counts at 120 DAS in the field trial were ^{10}log transformed before ANOVA. Sorghum grain yield data from the field trial and prevailing prices for sorghum grain, fertiliser and labour in Mali were used to perform a partial cost-benefit analysis and calculate the marginal rate of return (MRR) following the procedure as described (CIMMYT 1988).

Results

Greenhouse study at Wageningen University, the Netherlands

Two strigolactones, sorgomol and 5-deoxystrigol, were detected in all sorghum cvs (CGM-19/1-1, DouaG, Lina-3) at Rt 7.9 and 4.7 respectively (Fig. 1). The amount of strigolactones secreted as well as the ratio between the two strigolactones detected differed between the sorghum cvs (Fig. 2 A-B). CGM-19/1-1 secreted particularly high levels of sorgomol and much less 5-deoxystrigol while Lina 3 showed the reverse pattern. DouaG produced less sorgomol than CGM-19/1-1 and less 5-deoxystrigol than Lina-3. An increasing dose of DAP fertiliser strongly reduced the level of secretion of both strigolactones (Fig. 2 A-B).

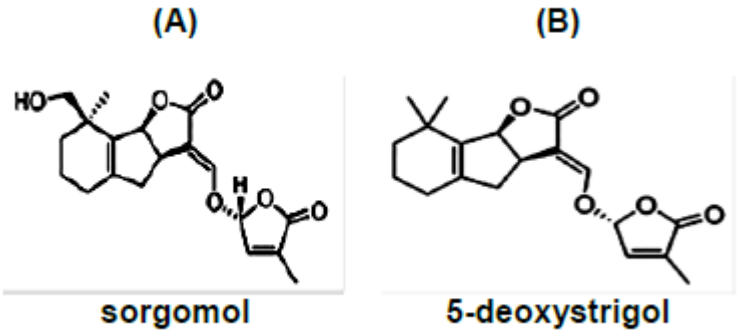

Fig. 1 Chemical structure of sorgomol (A), and 5-deoxystrigol (B) found in root exudates of sorghum cultivars.

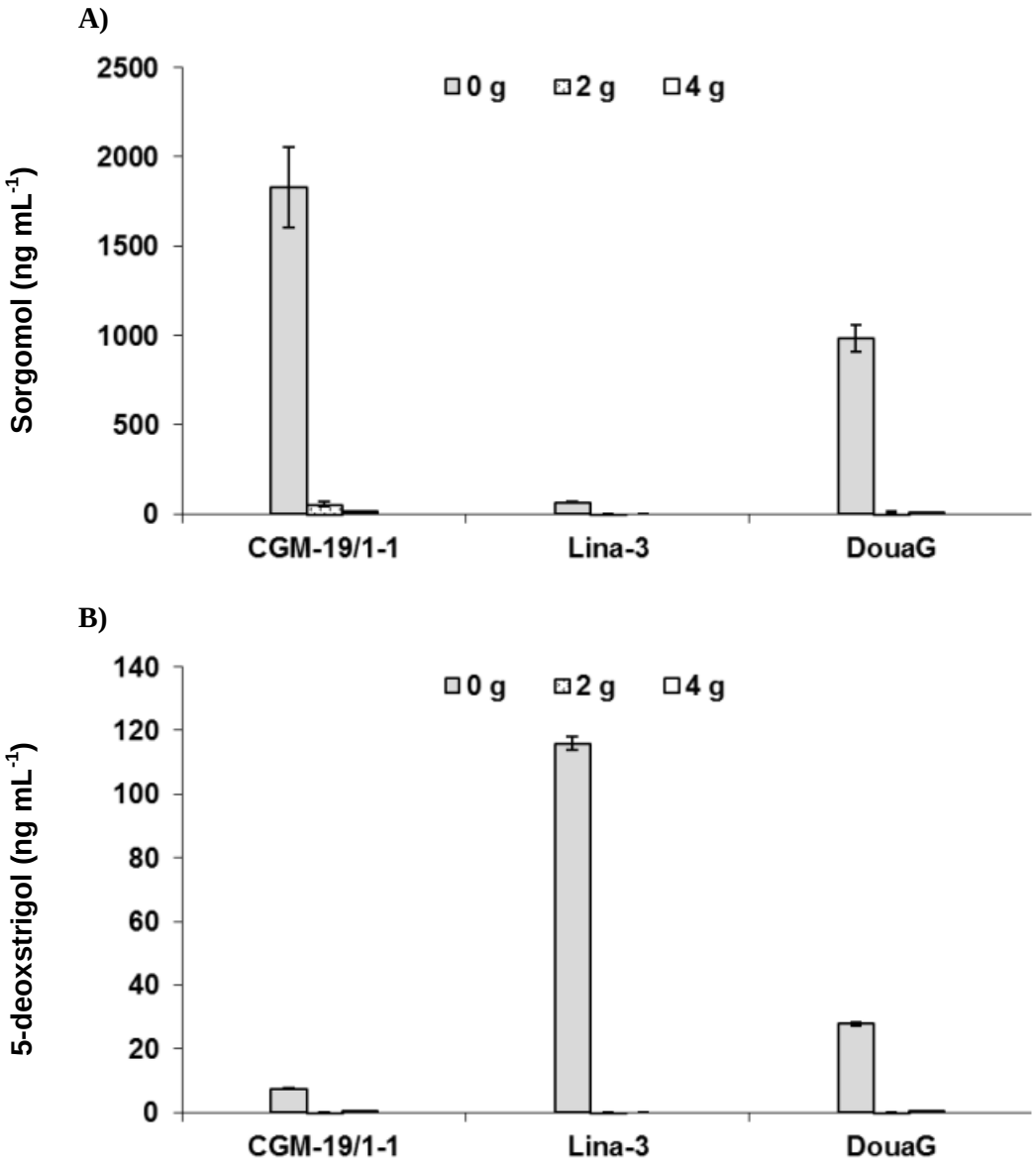

Fig. 2 Effect of microdosing of diammonium phosphate on secretion of sorgomol (A) and 5-deoxystrigol (B) in three sorghum cultivars. The purified root exudates were analysed using MRM-LC-MS (see Materials and methods). Bars represent means of amount of individual strigolactones (ng/mL) as determined by MRM-LC-MS in triplicate± SE (*n*=3).

In line with these results, application of sorghum root exudates collected from the control treatment (0 DAP) resulted in the highest *Striga* germination for all three cvs while an increasing dose of DAP reduced germination by up to 66-70% (Table 2; Suppl. Fig. 2). Similarly the control treatment (0 g DAP) resulted in maximum *Striga* emergence in the three cvs (Fig. 3) while increasing doses of DAP reduced emergence by 73% (Table 2; Suppl. Fig. 2). Maximum *Striga* emergence (62 plants per sorghum plant in CGM-19/1-1; 45 in Lina-3 and 23 in DouaG) occurred in the control treatment and microdosing of DAP reduced and delayed *Striga* emergence in all three cultivars (Suppl. Fig. 3). Similarly the highest *Striga* biomass occurred in the control treatment while microdosing of DAP

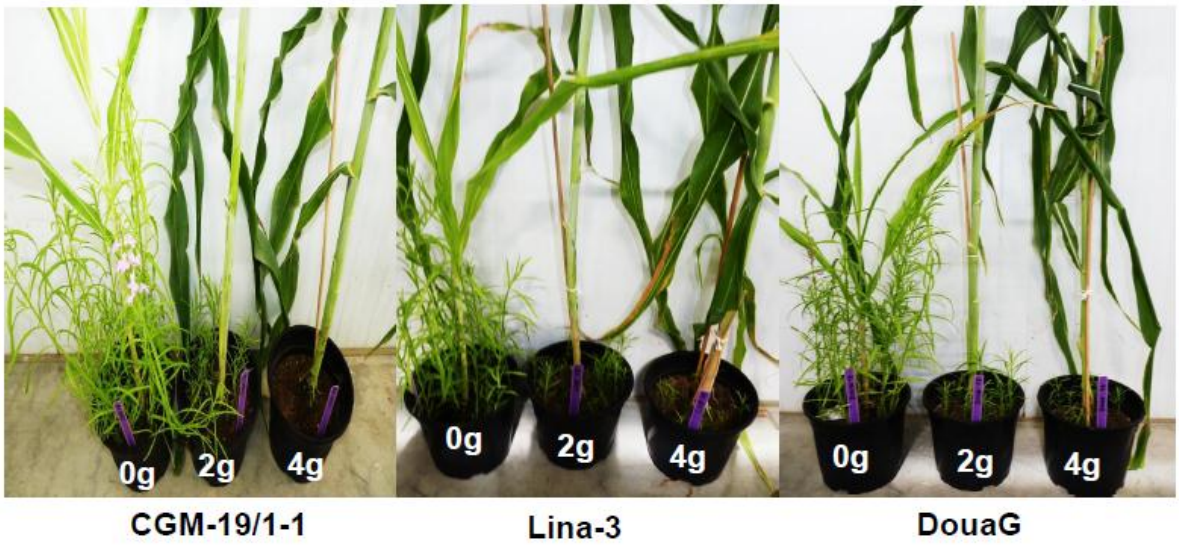

Fig. 3 Effect of diammonium phosphate microdosing on *Striga hermonthica* emergence at Wageningen University, the Netherlands. The emergence counted on individual sorghum host plant (*cvs.* CGM-19/1-1, Lina-3 and DouaG) at 0g DAP (62, 45, 23), 2g DAP (26, 26, 18), 4g DAP (17, 14, 12). Bars represent means ± SE (*n*=4).

Table 2 *Striga hermonthica* germination, emergence and dry biomass as influenced by DAP micro dosing in three sorghum cultivars in the pot trial under greenhouse conditions at Wageningen UR, the Netherlands

	DAP (g)	Germination (%)	Emergence (No.)	Dry biomass (g)
CGM-19/1-1				
	0	45±3.1 †	62±3.5†	6.0±0.3†
	2	29±1.3	26± 3.3	2.0± 0.4
	4	14±1.9	17± 1.2	0.2± 0.03
Lina-3				
	0	35±5.2†	45±4.8†	5.2±0.3†
	2	21± 2.5	26± 2.9	1.6± 0.8
	4	11± 1.3	14± 2.5	0.3± 0.1
DouaG				
	0	38±4.3†	23±1.5†	3.1±0.5†
	2	20±4.1	18±1.0	1.1±0.1
	4	13±1.6	12±1.4	0.3±0.1
Variety (*P*)		NS	<0.001	<0.001
DAP (*P*)		<0.001	<0.001	0.001
Variety X DAP (*P*)		NS	<0.001	<0.01
Linear		*** (-ve)	*** (-ve)	*** (-ve)
S.E.M‡		2.0	2.2	0.3
LSD 5% §		5.6	4.5	0.6
CV (%)		27	20	33

† Means±standard error *n*=4; §Least significant differences of means at *P* = 0.05 by ANOVA test; ‡ Standard error of difference of means; $^{**}P$ <0.01; $^{***}P$ <0.001.

reduced biomass significantly (Table 2). All the *Striga* infection parameters showed a significant negative linear response with an increasing dose of DAP fertiliser which was strikingly similar for the three cultivars (Table 2). Shoot dry weight and total dry biomass increased with microdosing of DAP while root weight and root:shoot ratio showed reverse trends and decreased with increasing dose of DAP (Table 3). Root:shoot ratio decreased from 3.6 to 0.9 for cv Lina3 and decreased from 6.9 to 0.6 for cv DouaG with increasing DAP fertilisation, with a significant interaction between DAP fertilisation and variety.

Table 3 Plant biomass (shoot/root) in various sorghum cultivars in response to DAP micro dosing in the pot trial under greenhouse conditions at WUR, Wageningen

	DAP	Shoot dry biomass	Root dry biomass	Total dry biomass	Root:shoot ratio
	(g)	(g)	(g)	(g)	
CGM-19/1-1					
	0	5.4±0.3†	19.6±0.6†	25.0±1.0†	3.6
	2	11.4±0.4	18.8±1.4	30.2±1.8	1.6
	4	18.2±0.7	16.8±1.4	35.0±2.2	0.9
Lina-3					
	0	4.1±0.2†	24.2±0.5†	28.3±0.7†	5.9
	2	18.5±0.3	20.4±1.0	38.9±1.3	1.1
	4	20.4±0.4	20.6±0.9	41.0±1.3	1.0
DouaG					
	0	2.7±0.2†	18.5±1.0†	21.2±1.2†	6.9
	2	11.8±0.6	15.8±1.1	27.6±1.7	1.3
	4	20.7±0.7	11.8±0.9	32.5±1.6	0.6
Variety (*P*)		<0.01	<0.01	<0.001	<0.001
DAP (*P*)		<0.001	<0.001	<0.001	<0.001
Variety X DAP (*P*)		<0.001	NS	NS	<0.001
Linear		*** (+ve)	** (-ve)	*** (+ve)	*** (-ve)
S.E.M‡		1.0	1.4	1.8	0.4
LSD 5% §		2.0	2.8	3.7	0.8

† Means±standard error *n*=4; ‡Standard error of difference of means; §Least significant differences of means at *P* = 0.05 by ANOVA test ; $^{**}P$ <0.01; $^{***}P$ <0.001.

Striga hermonthica emergence at ICRISAT Samanko research station, Mali

All the sorghum cvs exhibited a similar trend for *Striga* emergence in the field trial as in the pot trial performed in the green house, although with some variation (Table 4; Suppl. Fig. 4). That is, microdosing of DAP fertiliser significantly reduced *Striga* emergence and dry biomass among all sorghum cultivars for the application of 4 g of DAP fertiliser per hill, but the application of 2 g DAP fertiliser per hill led to an increase in *Striga* numbers with cv DouaG and an increase in *Striga* numbers and biomass with cv Lina-3. The control treatment (without DAP) induced maximum *Striga* emergence (20.4 plants m^{-2}) and dry biomass (33 g m^{-2}) with the susceptible variety CGM19/1-1. With this cultivar, microdosing with 4 g of DAP fertiliser per hill showed a considerable reduction of

Striga emergence to 1.1 plants m^{-2}, equal to 84% reduction. Similarly, *Striga* biomass decreased from 33 g m^{-2} in the control treatment to 2.8 g m^{-2} with 4 g of DAP fertiliser, which is a reduction of 80%. In addition to this, DAP microdosing affected sorghum grain and stalk yield significantly and both showed a positive trend with the DAP rate among all cultivars. Sorghum grain yield increased considerably for all cultivars when comparing the application of 2 and 4 g DAP per hill to the control treatment, with the increase ranging between 43 and 142% for DouaG and CGM19/1-1, respectively.

Table 4 *Striga hermonthica* emergence and dry biomass and sorghum stalk and grain yield as influenced by DAP micro dosing in three sorghum cultivars under field conditions in Mali

	DAP (g)	Emergence (No. m^{-2})	Dry biomass (g m^{-2})	Stalk yield (t ha^{-1})	Grain yield (t ha^{-1})
CGM-19/1-1					
	0	20.4±2.8†	32.8±7.5†	4.2±0.8†	0.8±0.2†
	2	9.8±5.4	11.1±7.0	7.6±0.8	1.7±0.2
	4	7.1±3.1	8.1±4.1	8.4±1.4	2.0±0.3
Lina-3					
	0	6.9±3.3†	14.3±7.1†	7.4±0.9†	1.5±0.3†
	2	7.9±2.0	9.4±3.6	10.0±0.9	2.1±0.3
	4	1.4±0.4	2.8±1.2	10.2±0.7	2.2±0.2
DouaG					
	0	2.4±1.1†	5.4±3.8†	7.3±1.3†	1.4±0.3†
	2	4.2±2.6	10.1±5.6	10.2±1.4	2.0±0.2
	4	1.4±1.3	3.0±2.4	12.4±1.1	2.1±0.2
Variety (*P*)		0.007	0.031	0.004	NS
DAP (*P*)		<0.001	0.016	<0.001	<0.001
Variety X DAP (*P*)		NS	NS	NS	NS
Linear		* (-ve)	* (-ve)	*** (+ve)	*** (+ve)
S.E.M‡		1.6	2.9	0.1	0.6
LSD 5% §		4.7	8.5	1.8	0.4
CV		10.6	36.1	14.7	7.2

† Means±standard error *n*=4; ‡Standard error of difference of means; §Least significant differences of means at *P* = 0.05 by ANOVA test; $^{**}P$ <0.01; $^{***}P$ <0.001

Partial cost-benefit analysis and marginal rate of return

The total additional cost for the application of 4 g DAP fertiliser per hill is about $93 per hectare, while the cost of application of 2 g DAP is $50 per hectare (Table 5). Among DAP micro-dosing treatments, the application of both 2 and 4 g DAP per hill gave higher net benefits as compared with control treatment. Application of 2 g DAP resulted in higher net benefit in *cv.* Lina-3 and DouaG cultivars ($641-682 ha^{-1}) while 4 g DAP showed more benefit in *cv.* CGM-19/1-1 only ($594 ha^{-1}). When considering the marginal rate of return (MRR), application of 2 g DAP per hill was found superior to the application of 4 g DAP per hill in all three sorghum cultivars with an MRR of 318, 366 and 523% for cvs DouaG, Lina-3 and CGM-19/1-1, respectively (Table 6).

Table 5 Partial cost benefit analysis on the basis of sorghum grain yields from the field trial in 2010 at ICRISAT-Samanko, Mali and current prices for DAP fertilizer, labor and sorghum grain

Sorghum cultivars	CGM-9/1-1			Lina-3			DouaG			Remarks
Diammonium phosphate	0g	2g	4g	0g	2g	4g	0g	2g	4g	g
Grain yield (kg/ha)	830	1740	2010	1460	2140	2210	1410	2020	2070	Kg ha^{-1}
10% less than actual yield	83	174	201	146	214	221	141	202	207	Kg ha^{-1} (at farmer's level)
Adjusted yield	747	1566	1809	1314	1926	1989	1269	1818	1863	Kg ha^{-1}
Gross income	284	595	687	499	732	756	482	691	708	Sorghum price $38.04 per 100kg bag
Fertilizer amount used	0	62.5	125	0	62.5	125	0	62.5	125	(kg ha^{-1})
Cost of fertilizer	0	41	81	0	41	81	0	41	81	DAP price $ 32.61 per 50kg bag
Fertilizer application cost	-	9	12	-	9	12	-	9	12	$ 3 man day (3 and 4 days for 2 g and 4 g DAP)
Cost that vary	0	50	93	0	50	93	0	50	93	$ ha^{-1}
Net benefit	284	545	594	499	682	663	482	641	615	$ ha^{-1}

Table 6 Calculation of the marginal rate of return for the different cultivar times microdose treatment based on the field trial 2010 at ICRISAT-Samanko, Mali

	Rate (g)	[a] Cost ($ ha^{-1})	Net Benefit ($ ha^{-1})	[b] Marginal Cost ($ ha^{-1})	[c] Marginal Net Benefit ($ ha^{-1})	[d] Marginal Rate of Return (%)
	0	0	284	-	-	-
CGM 19/1-1	2	50	545	50	262	523
	4	93	594	43	49	113
	0	0	499	-	-	-
Lina-63	2	50	682	50	183	366
	4	93	663	43	-19	-44
	0	0	482	-	-	-
DouaG	2	50	641	50	159	318
	4	93	615	43	-26	-60

[a] Cost for particular treatment ($)
[b] The increase in variable cost which occurs in changing from one production alternative to another
[c] The increase in net benefit which can be obtained by changing from one production alternative to another
[d] dividing marginal net benefit with marginal cost (in %)

Discussion

Parasitic plants generally prevail on nutrient deficient soils and many studies have reported a decrease in *Striga* infection upon application of N and P (Gworgwor & Weber 1991; Kim et al. 1997; Adagba et al. 2002). A direct or indirect relationship between the presence of mineral nutrients and *Striga* infection has been suggested in previous studies (Cechin & Press 1993; Gacheru & Rao 2001; Showemimo et al. 2002; Pageau et al. 2003; Kamara et al. 2007). The germination of *Striga* seed is associated with the secretion of germination stimulants by host plants which ultimately depend upon

the nutrient status of the soil (Jamil et al. 2011a). It has been demonstrated that under N and P deficiency, host plants secrete high amounts of germination stimulants into the rhizosphere while supply of sufficient N and P reduces this secretion (Ayongwa et al. 2006; Yoneyama et al. 2007; Lopez-Raez et al. 2008).

The three sorghum cultivars secreted the highest amount of strigolactones in the treatment without DAP fertiliser and the secretion decreased with increasing rates of application of DAP fertiliser (Fig. 2). The secretion of these strigolactones in the treatment without DAP fertiliser caused maximum germination and emergence of *Striga*. The sorghum *cv.* DouaG showed some resistance with less strigolactone production and *Striga* infection while *cv.* CGM-19/1-1 appeared highly susceptible with high sorgomol secretion and *Striga* infection in the control treatment. Low germination stimulant producing sorghum cultivars have been reported before to exhibit resistance to *Striga* in the field and have been tested and adopted in many African countries where they were found effective against *Striga* (Hess et al. 1992; Ejeta 2005; Ejeta 2007).

The results in the greenhouse pot trials are similar to the results in the field trials. All the sorghum cvs showed statistically significant reduction in *Striga* emergence and biomass and an increase in sorghum grain and stalk yield with the highest dose of 4 g DAP per hill. However, it must be noted that the intermediate dose of 2 g DAP per hill that resulted in higher sorghum grain and stalk grain, also resulted in higher *Striga* emergence with the cvs DouaG and Lina3 and higher *Striga* biomass with cv Lina3. DAP microdosing also affected the tolerance of sorghum plants to *Striga* attack, as shown by increased yields under higher levels of *Striga* emergence for cvs Lina3 and DouaG (compare 0 and 2 g DAP per hill in Table 4). It is suggested that in addition to a reduction in *Striga* infection, microdosing with DAP fertiliser has an additional positive effect of enhancing the tolerance of sorghum to *Striga* infection. This stresses the importance of combining microdosing with other control measures to develop integrated *Striga* management.

A similar study on maize with field trials in Kenya, showed the same trend of *Striga* reduction with an increase in N and P fertilisation (Jamil et al. 2011b), but particularly under greenhouse conditions. The results under field conditions in Kenya were less consistent as compared with the results of the current study on sorghum in Mali. The difference in fertiliser type and mode of application of fertiliser may have caused this difference. The fertilisers used in Kenya were Urea and Triple Super Phosphate (applied as side dressing in the row) while in Mali it was diammonium phosphate (applied in holes near the seed as microdosing). The N in Urea fertiliser might be lost more quickly due to volatilisation, denitrification and leaching as compared with the slow releasing DAP. Similarly, P in TSP may adsorb on soil particles more quickly than DAP (Tisdale et al. 2004). Finally, soils in West-Africa are generally more sandy and suffer less from acidic pHs as the soils in central-Africa. Hence, P availability after fertiliser application in Mali may be much better than the availability in Kenya.

DAP micro-dosing (2 or 4 g per hill) increased grain and stalk yields of sorghum considerably, up to a maximum of 142%. Moreover, higher economic benefits were obtained in all microdosing treatments as compared to the control. Application of 2 g DAP appeared the best treatment with the highest net benefit and the highest MRR for all sorghum cultivars. The lower or even negative MRR for the application of 4 g DAP per hill indicates that this dose is not economically viable even though in agronomic terms, namely *Striga* reduction and sorghum yield increase, the treatment of 4 g DAP per hill is the best. It should however be noted that we did not consider stalk yield in the cost-benefit analyses. The increased stalk yield has an additional value as fodder, building material and source of fuel from the stalks, which could make the 4 g DAP rate economically more attractive. The large increase in MRR between the control treatment and the application of 2 g DAP per hill suggests that there may also be room for decreasing the dose applied per hill to less than 2 g DAP per hill. The results obtained in the present study need to be shared with farmers and confirmed over several years through participatory validation in farmers' fields. If farmers are properly informed and convinced by having seen the technique and its effects with their own eyes, farmers will most likely chose the option of 2 g DAP per hill as this requires less initial investment.

Although the MRR is high and it makes sense to invest in microdosing DAP at sowing, this technique may still prove to be a problem for farmers in the semi-arid region in sub-Saharan Africa. A number of environmental and socio-economic factors can affect the effectiveness of microfertilising technology. Erratic rainfall and drought, typical events for the regions where sorghum is grown, can reduce plant nutrient uptake after application of fertiliser leading to limited yield gains and thus higher risk for the farmer to use fertiliser. It is also crucial to sow as soon as possible in the season and immediately after rain because nutrients are lost quickly and the soil will quickly dry out, leading to drought stress if rainfall after sowing is erratic. In regions where early sowing is essential, microdosing is a constraint because with the same labour, the farmer can only sow half of the area compared to no application of fertiliser (Aune et al. 2007). A study of Aune *et al*. (2007) has shown that mixing seeds with NPK or DAP fertiliser in a 1:1 volume ratio, leading to the extremely low dose of about 0.3 g per hill was economically more feasible than applying 6 g per hill. In addition, mixing seeds with fertiliser eliminates the labour constraint as the sowing would be almost as fast as normal sowing without fertiliser. The results of the current study do not contest, nor confirm the findings of Aune *et al*. (2007), but suggest that studying the effects of lower doses of fertiliser makes sense in socio economic terms and considering the effectivity of *Striga* control. Microdosing of DAP fertiliser could prove very effective for *Striga* control and increasing the yields of grain and stover of sorghum. Due to low cost, low amount of fertiliser used and high efficiency, this technology may be attractive for poor farmers growing sorghum on infertile soils, often in combination with *Striga* infestation.

The outcomes of the present study indicate that it is possible to enhance sorghum production with relatively small investments in fertiliser in Africa. It is suggested that use of DAP fertiliser as a

microdose aids in reducing *Striga* infection due to a reduction in the secretion of strigolactones by the host plant while at the same time improving sorghum development and yield under *Striga* infection. Microdosing of DAP fertiliser should, however not be considered as a single option for *Striga* control, but should be combined with other options such as intercropping and rotation with legume crops and the use of appropriate resistant cereal cultivars to achieve integrated *Striga* and soil fertility management.

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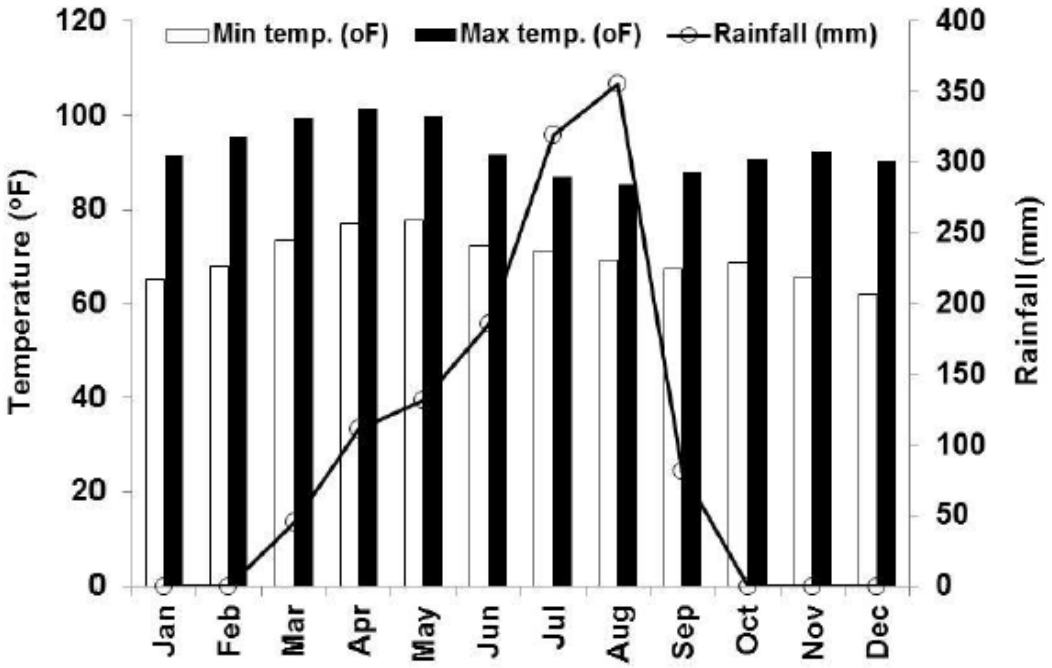
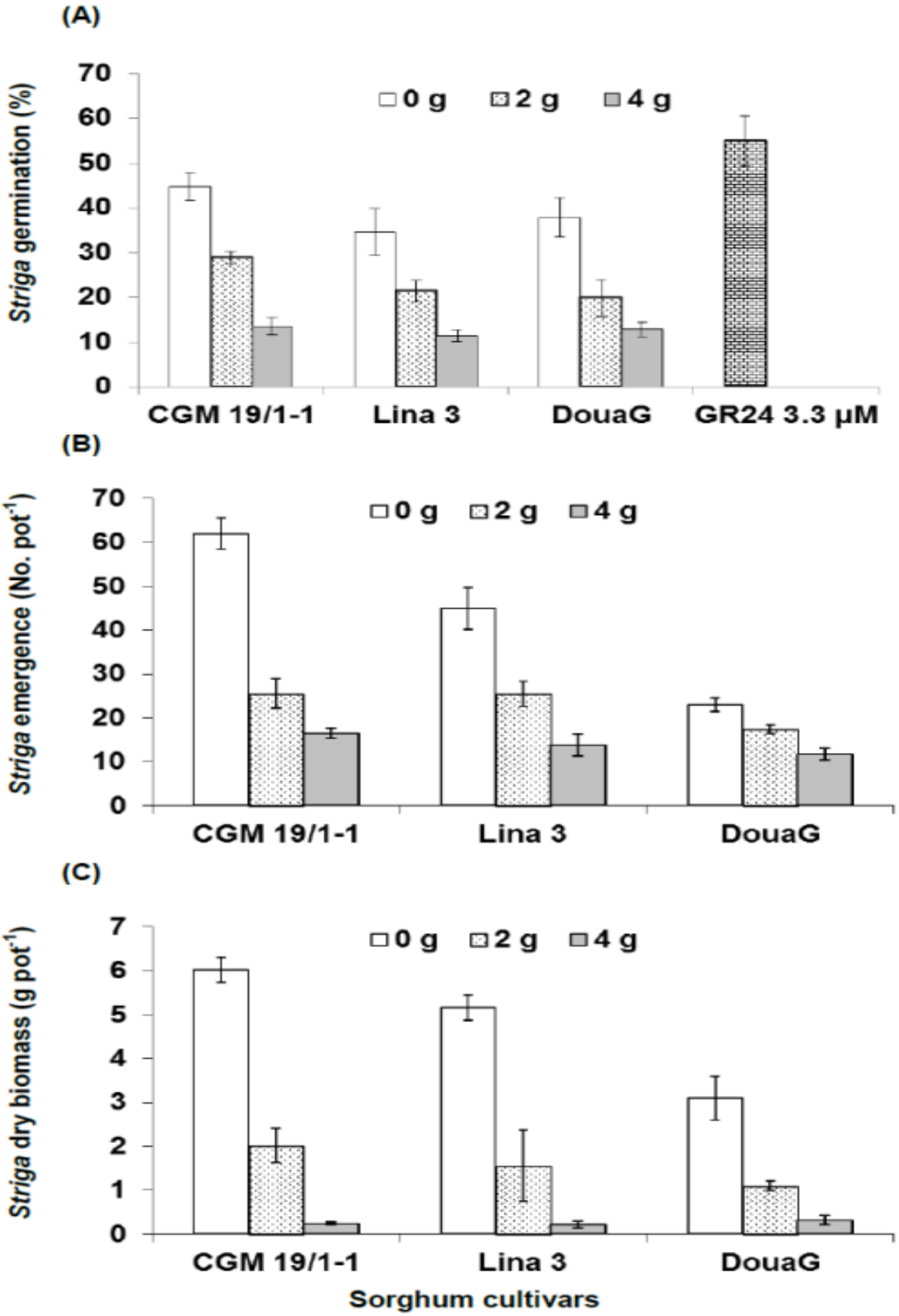
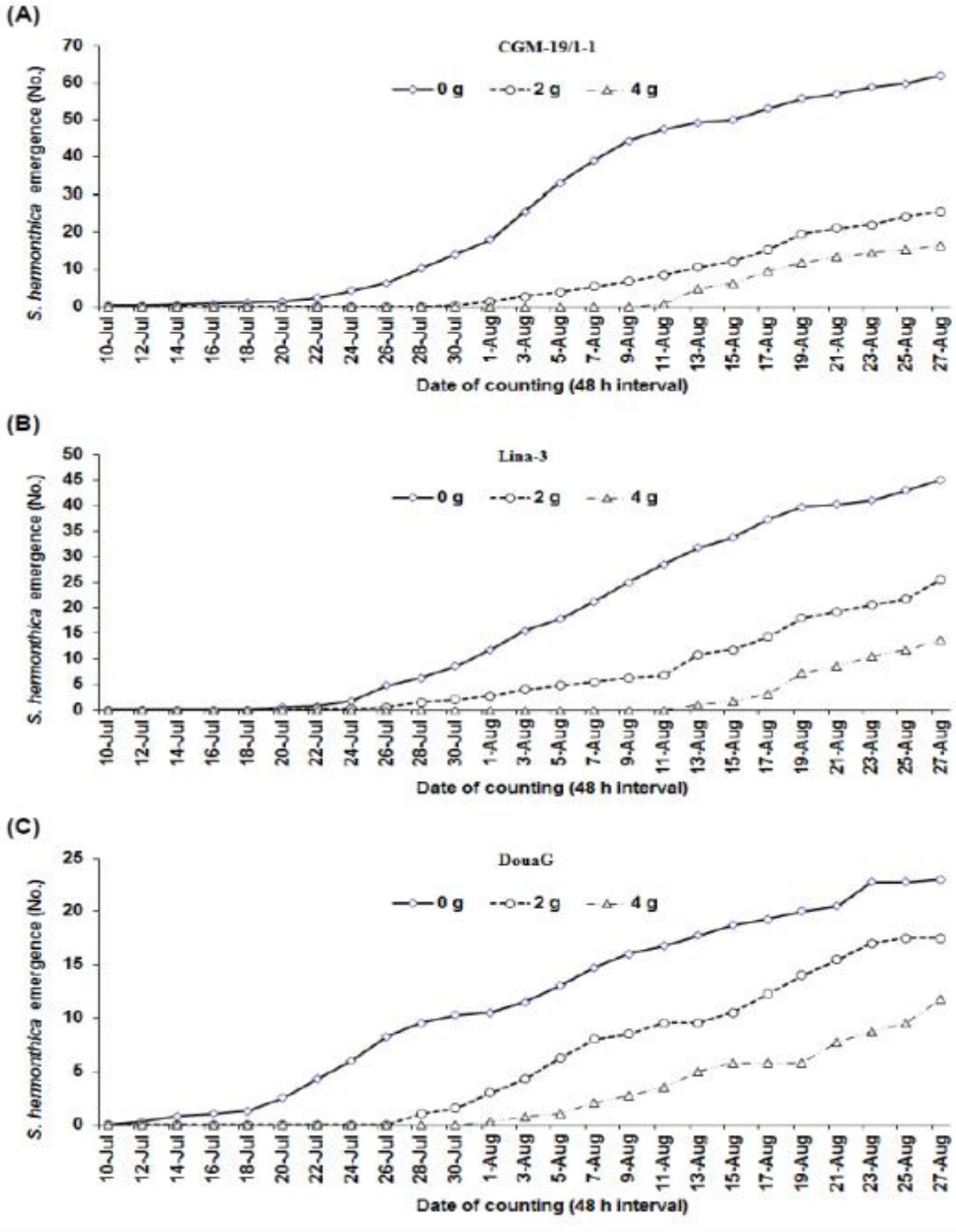


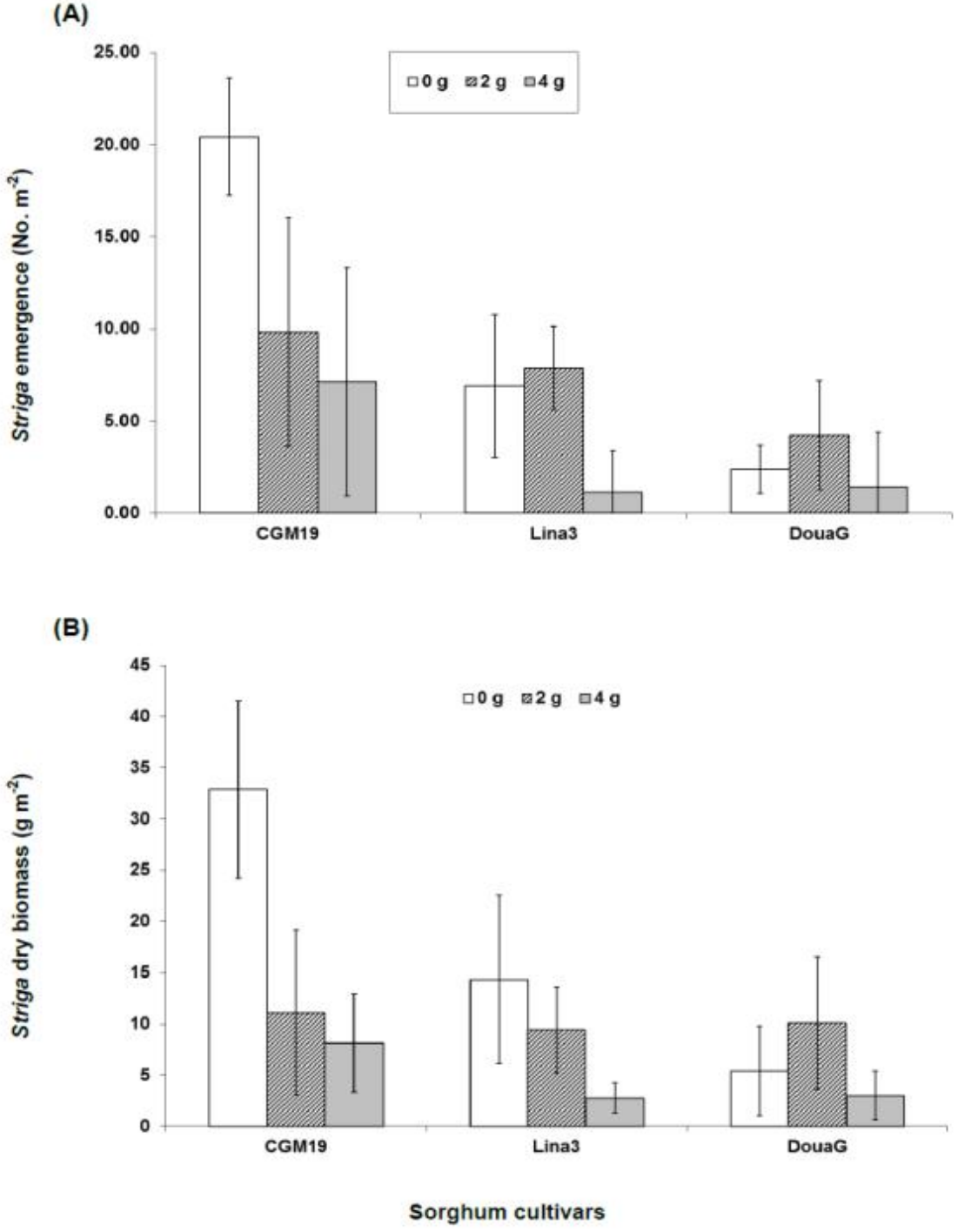
Suppl. Fig. 1 Meteorological data of field experimental site (Mali) during 2010 obtained from Mali Meteorological Department. The bars represent average maximum and minimum temperature (in ^{o}C) while line diagram represent monthly average rainfall (in mm).



Suppl. Fig. 2 Effect of diammonium phosphate microdosing on *Striga hermonthica* germination (A), emergence (B) and dry biomass (C) at Wageningen University, the Netherlands. The *Striga* seeds germination was counted after application of sorghum root exudates. *Striga* emergence was counted on sorghum host plant up to 10 weeks of sowing. Bars represent means ± SE (*n*=4).



Suppl. Fig. 3 Periodic emergence of *Striga hermonthica* with two days interval at Wageningen University, the Netherlands as influenced by DAP microdosing in three sorghum cultivars.



Suppl. Fig. 4 Effect of diammonium phosphate microdosing on *Striga hermonthica* emergence (A) and dry biomass production (B) at Samanko research station, Mali. *Striga hermonthica* emergence were counted at 120 days after sowing and dry biomass at 90, 120, 150 days after sowing. Bars represent means ± SE (n=4).

Chapter 8

General discussion

The present thesis describes the possibilities to target the carotenoid derived underground signalling molecules called "strigolactones" to improve the control of the parasitic weed *Striga*. The strigolactones were originally identified as compounds that trigger germination of parasitic plant seeds (Cook et al. 1966; Xie et al. 2010; Yoneyama et al. 2010) and stimulate symbiotic mycorrhizal fungi (Akiyama et al. 2005). Later on strigolactones were also found to be involved in tillering/ branching regulation (Gomez-Roldan et al. 2008; Umehara et al. 2008) as an adaptive strategy under adverse P stress (Umehara et al. 2010; Kohlen et al. 2011a). Recently strigolactone involvement in the regulation of root architecture in interaction with other plant hormones like auxin has been demonstrated (Koltai et al. 2010; Kapulnik et al. 2011a; Kapulnik et al. 2011b; Ruyter-Spira et al. 2011).

In this thesis we show that the biosynthesis of this class of compounds is regulated according to the status of soil fertility and nutrient availability. Indeed the struggle to obtain nutrients for survival is the main driving force involved in strigolactone secretion by the host plant into the rhizosphere. When host plants sense a deficiency of mineral nutrients, particularly phosphorus and nitrogen, they start signalling to AM fungi by increasing their strigolactone production and exudation into the soil (Bouwmeester et al. 2007; Lopez-Raez and Bouwmeester 2008). Symbiosis with AM fungi plays an important role in mineral nutrient uptake, especially of phosphorus, in over 80% of land plants (Akiyama and Hayashi 2006; Harrison 2005). Host plants release strigolactones to induce extensive hyphal branching in AM fungi (Akiyama et al. 2005; Nagahashi and Douds 2000). This pre-symbiotic growth facilitates root contact, penetration and colonization by the fungus (Buee et al. 2000). However, this 'branching factor' signalling sometimes results in a parasitic, rather than a symbiotic, interaction when seeds of parasitic plants perceive these signalling molecules. Due to very limited food reserve in the seeds, the parasitic plants completely rely on a host for their survival and they have evolved this host detection mechanism using the signalling molecules intended for the AM fungi.

As much of the damage to the host occurs belowground during the early stages of parasitism, the traditional control measures applied after emergence are not very effective. Therefore, novel, efficient and feasible control strategies, targeted at pre attachment and emergence processes, need to be developed. Since germination of *Striga* depends upon germination stimulants or strigolactone signalling in the rhizosphere, we hypothesized that such dependency on host derived signals could be used to develop better control measures (Bouwmeester et al. 2003; Lopez-Raez et al. 2008). Control methods developed on the basis of secretion of "germination stimulants", the first critical step in the

lifecycle of *Striga*, can be more effective and efficient, because these methods would avoid the early *Striga* damage (Parker and Riches 1993; Eplee and Norris 1995; Bouwmeester et al. 2003). So the objective of the present Ph.D. dissertation is to explore the relationship between host strigolactone production and *Striga* infection in cereals and to elucidate how a reduction in strigolactone production can be obtained in order to control *Striga*. In this thesis we approached this in three different ways. As mentioned above the strigolactones are derived from the carotenoid biosynthesis pathway, so we explored the possibility to reduce strigolactone formation through the use of carotenoid biosynthesis inhibitors (Jamil et al. 2010). In several plant species it was shown that there is genetic variation in quantity and composition of strigolactone production (Awad et al. 2006; Yoneyama et al. 2008) and we explored that in this research for rice (Jamil et al. 2011b; Jamil et al. 2011c). Finally, we investigated the relationship with soil fertility that is described above (Jamil et al. 2011a). All these aspects can possibly be deployed as practical control measures to lower *Striga* infection at the pre-attachment phase.

Use of carotenoid inhibitors

Considering the carotenoid origin of the strigolactones (Matusova et al. 2005), it was postulated that the (mild) inhibition of carotenoid biosynthesis by carotenoid inhibitors, particularly in the roots, could lead to a decrease in the production of strigolactones. In chapter 2 it was demonstrated that the application of carotenoid inhibitors like fluridone, norfluarzon, clomazone and amitrole to plants can indeed affect *Striga* germination and attachment by reducing the strigolactone concentration in the rhizosphere. The results indicated that application of carotenoid inhibitors significantly decreased strigolactone production, *Striga* germination and *Striga* infection without affecting growth and development of the host plant. The outcome of the present study can be used to design a *Striga* control strategy based on the application of strigolactone biosynthesis inhibitors. Due to the very low required dosage of these commercially available herbicides, an effective and practically viable *Striga* control technology for poor farmers in the African continent can potentially be developed. In fact the present study on use of carotenoid inhibitor could be a first step towards indirect herbicidal control of *Striga* due to reduced strigolactones secretion. Since this is a basic, preliminary study, carried out under greenhouse conditions, for the development into practical application, a field study is required especially on the choice of a suitable application method, right concentration, timing and number of applications.

Genetic variation

Genetic variation in the quantity and quality of strigolactone production has been suggested as selection criterion for pre-attachment resistant genotypes in sorghum (Ejeta 2007). In this thesis we decided to study the presence of genetic variation in strigolactone production in rice. Hereto we studied a group of rice cultivars called "NERICAs" (Chapter 3) and a series of rice varieties collected from all over the world (Chapter 4). Africa Rice Center and partners have developed a collection of inter-specific upland rice cultivars, named NERICA (New Rice for Africa). These cultivars were developed with the aim to combine the high yields from the Asian rice species *O. sativa* (WAB 56-104, WAB 56-50 and WAB 181-18) with the ability of the African species *O. glaberrima* (CG 14) to resist local stresses (Jones et al. 1997a, b). To date, 18 of such interspecific upland cultivars are available to rice farmers. They are popular among rice farmers in the region and partly responsible for the recent increase in rice area under rain-fed upland conditions (Rodenburg et al. 2006c; Balasubramanian et al. 2007; Wopereis et al. 2008). NERICAs have a high yield potential and short growth cycle and offer a welcome relief to Africa's rice farmers. The results of the present study showed that there was significant variation among the NERICA cultivars and their parents for strigolactone production and *Striga* germination. The cultivars NERICA 1 and CG 14 showed resistance against *Striga* due to the least production of strigolactones while NERICAs 7 and 8 appeared the most susceptible cultivars, secreting a high amount of strigolactones and stimulating a high percentage of *Striga* seed germination. Across all cultivars and parents, there was a positive relationship between the amount of strigolactones in the exudate and *Striga* infection parameters. So it can be presumed that the amount of strigolactone production of a rice cultivar can explain the level of resistance against *Striga.* A relatively low production of strigolactones results in a lower percentage of *Striga* germination, contributing to a more resistant phenotype. In the NERICAs, strigolactone production was also found to negatively correlate with tiller numbers. Indeed strigolactone regulation of rice tillering has been already described in recent studies (Umehara et al. 2008; Umehara et al. 2010; Wang and Li 2011). The correlation between tillering, strigolactones and *Striga* infection in NERICA cultivars suggests that higher-tillering cultivars have better *Striga* resistance due to lower strigolactone production. Keeping this aspect in mind, we decided to conduct an independent study to see detailed genetic variation for rice tillering and strigolactone production and to link this feature with *Striga* infection in a range of rice cultivars from all over the world (Chapter 4). Interestingly, the low-tillering rice cultivars IAC 165 and IAC 1246 produced high amounts of strigolactones and high *Striga* infection. In contrast to this, high-tillering rice cultivars such as Super Basmati, TN 1 and Anakila exhibited low production of strigolactones and low *Striga* infection. A positive correlation between strigolactones and *Striga* infection and a negative relation with tillering further strengthened the assumptions that genetic variation for strigolactone production is reflected in tillering and *Striga*

infection. These results prove that genetic variation in strigolactone production could lead to variation in tillering capacity and this phenomenon could be used to select resistant rice cultivars against *Striga* infection. So, the tillering potential of rice turns out to be an important marker for the plant's susceptibility to *Striga* infection, and selection of suitable, high-tillering cultivars with less strigolactones might be a useful strategy to reduce *Striga* damage. Moreover, these findings suggest the existence of large genetic variation for strigolactone production among varieties that could be a good basis for breeding against strigolactones and hence for improved *Striga* resistance.

Soil fertility improvement

The links between *Striga* germination, germination stimulant production and the plant's nutritional status have been proposed and discussed in several studies (Yoneyama et al. 2007a; Lopez-Raez et al. 2008; Jamil et al. 2011a). As the incidence of *Striga* is negatively correlated with soil fertility, addition of N and P nutrients will result in lowering *Striga* infection (Cook et al. 1966; Kim and Adetimirin 1997; Kim et al. 1997a). Our Lab results show that the nutrient deficiency caused maximum strigolactone production and *Striga* germination while increasing doses of N and P reduced the amount of strigolactones in the exudates and *Striga* infection at the same time. A positive relationship between the amount of strigolactones in the exudates and *in vitro Striga* germination under varying nutrient levels further confirm the links between strigolactones, *Striga* infection and soil fertility (Chapters 5, 6, 7). The effect of nutrient deficiency on strigolactone production and *Striga* infection in rice, maize and sorghum suggests that fertilizers can play a vital role in reducing germination stimulant production and *Striga* infection in these cereals. The field study at Kenya (maize) and Mali (sorghum) in parallel also showed suppression of *Striga* infection and an increase in grain yield by fertilizer application. The application of Urea and triple super phosphate (TSP) in maize and microdosing of diammonium phosphate (DAP) in sorghum resulted in higher net profits as compared with the control treatments. However, the field results - especially on *Striga* infection in maize in Kenya – were less consistent than the greenhouse study or the sorghum field study in Mali. In maize, field application of P in the form of TSP did not show as much reduction of strigolactone production and *Striga* infection as in the greenhouse, where a strong negative correlation between P levels and strigolactone production and *Striga* infection was observed. Soil analysis of the Kenyan fields showed that despite the application of TSP there was a very limited amount of available P. Lack of organic matter, variation in soil pH, and P adsorption due to acidic reaction of soil has possibly resulted in low P availability to the maize host in the field in Kenyan soils. Furthermore, the type of fertilizer and the method of application may affect nutrient availability to the host and hence *Striga* infection. Indeed, in fertilizers trial in Mali, microdosing of DAP fertilizer to sorghum proved very efficient against *Striga*. So here we can say DAP microdosing in small holes is more effective against

Striga than side dressing field application of Urea/TSP. Since DAP is a slow-release fertilizer which was applied near the host roots, the sorghum plants in Mali could probably obtain nutrients easier and for a longer period of time, while the Urea and TSP in maize of Kenya perhaps got lost or adsorbed quickly due to instability or soil reaction. It is clear that all factors contributing to fertilizer use efficiency should be considered before formulating and recommending a fertilizer strategy for *Striga* control in the African fields. Nevertheless the outcomes of these studies show that the use of fertilizers can help to reduce *Striga* infection – and hence to improve cereals yields in Africa - and that this is at least partly due to the reduced secretion of germination stimulants into the soil due to improved nutrient availability.

Implications

The first impression from the present thesis findings is that the inherent potential of the host plant for strigolactone production and pre-attachment resistance can be employed as one of most efficient and effective ways of *Striga* reduction. Low strigolactone producing cereal cultivars could be developed or selected for and introduced to African famers. Such varieties could prove to be a good way to control *Striga* without much additional cost for fertilizer or herbicide. Indeed, in the present studies rice cvs like IAC 165 and NERICA 7 showed 100-200 fold higher production of 2'-epi-5-deoxstrigol and orobanchol than TN 1 or CG 14. Also in sorghum, *cv* CGM-19-/1-1 showed much higher production of sorgomol and 5-deoxystriogl than DouaG. So there is a large genetic variation in the levels of strigolactone production which could be used to develop varieties with decreased *Striga* germination and attachment. Due to the involvement of strigolactones in tillering regulation, however, low strigolactone producing hosts may display higher tillering (Chapter 3, 4). In this case this resistance breeding strategy might face some problems if African farmers have a preference for mono or low tillering varieties. So local needs and preferences should be considered before introducing this strategy. Other options like the use of carotenoid inhibitor herbicides or fertilizer application could also be adopted but all these depend upon prevailing socio-economic or edaphic situations and will result in lower strigolactone production which not only affects *Striga* germination but also likely host tillering. Moreover, the *Striga* control strategies based on low strigolactone production might disturb AM fungal symbiosis of the host due to reduced hyphal branching in the root zone and this could result in less efficient mineral nutrient acquisition by the host plant. All this could possibly be solved by sophisticated strigolactones analytical approaches as discussed below.

If we realize all these important metabolic and physiological roles of strigolactones in the host plant life, it is not recommended that strigolactone biosynthesis should be stopped completely in the host plant. So due attention should be paid to the link between structural requirements of strigolactones for germination stimulation in parasitic weeds, hyphal branching in AM fungi or

tillering regulation in the host plant (Akiyama et al., 2010; Zwanenburg et al. 2009). In the present thesis we also observed genetic variation in strigolactone composition in addition to quantity produced by different rice and sorghum cultivars. It would be of interest to test whether AM fungi and *Striga* are triggered by exactly the same or different types of strigolactones and consequently if it would be an option to identify cultivars that produce the type of strigolactones that stimulate AM fungi without triggering *Striga* germination. Akiyama and co-workers (2010) confirmed that some strigolactones - especially 5-deoxystrigol and orobanchol - induced AMF hyphal branching more actively than others such as strigol and sorgomol. So the strigolactone stereochemistry can influence stimulant activity significantly. Combining molecular information could lead to a model for the design of synthetic strigolactones. The C- and D-rings connected with an enol ether bond in the strigolactone molecule were identified to be the essential structure for germination stimulation (Mangnus and Zwanenburg 1992), and both constitution and configuration of the stimulants were also shown to influence the biological activity greatly (Yoneyama et al. 2009 , Zwanenburg et al. 2009). The difference in structure within strigolactones might lead to their specific response in the rhizosphere as well as in the host plant. Thus selective reduction in the amount of specific strigolactones acting as germination stimulants could raise resistance to parasitic weeds whereas other strigolactones leading to hyphal branching or tillering regulation could be allowed to remain for the benefit of host plant-rhizosphere interactions. This knowledge can be useful for plant breeders to select cultivars with the desired types of strigolactones for maximum AMF hyphal branching and lower production of only those strigolactones which act as germination stimulants. For example, the rice cultivars N-15 and Dullo in the present studies have been identified as high producers of orobanchol with less *Striga* germination and intermediate tillering. These cultivars could be further tested for their activity in AMF hyphal branching. Still very little knowledge is available on the structural requirements of strigolactones for AM fungi hyphal branching, *Striga* seed germination and tillering/branching inhibition. Identification and (bio)synthesis of new natural and synthetic stimulants to establish the molecular connection between strigolactones and their biological activity as germination stimulants, as branching factors and as tillers/shoot branching inhibitors can be an important task and challenge to be done in the future.

The results from the present thesis studies offer the potential to develop a package of *Striga* control technologies targeted at the pre-attachment stage. Their practical implication, however, will still require further research before general recommendations for the farmers can be made. For example, the experiments with fertilizer application carried out under lab and greenhouse conditions used short term nutrient stress only. Therefore, the results from these studies must be interpreted with care and need to be confirmed in field trials before *Striga* control strategies can be formulated and recommended for agricultural purposes. The results of the two field trials, conducted in actual field conditions in Kenya and Mali showed a fair reduction in *Striga* resulting in an increase in yield.

However, the socio-economic factors like farmer's poverty and affordability to purchase costly fertilizers or carotenoid inhibitors, limited availability of inputs especially resistance germplasm and less accessibility of farmers, disfunctional markets, lack of information and illiteracy can affect practical field application of these *Striga* control strategies. Moreover the choice of a suitable application method, right dose or concentration, timing and number of applications is very important for developing such *Striga* control technology for the farmers. In addition to this the soil physico-chemical properties, pH, water logging and salinity, structure, texture, organic matter and N contents are likely very different in the field which could greatly affect *Striga* infection. Phosphorus adsorption on soil particles and N losses due to denitrification, volatilization and leaching might affect nutrient availability and hence *Striga* infection. Similarly, the *Striga* seed bank in the soil, time and method of host planting, erratic rain fall and temperature fluctuation can reduce or enhance *Striga* seed germinative ability and infection.

Prospect for Integrated *Striga* Management (ISM)

It is clear that a single *Striga* control option cannot result in a durable solution. In order to achieve sustainable strategies for *Striga* management, several control options should be adopted and adjusted on the basis of individual cropping systems, local needs and preferences. Control strategies targeted at the early developmental phases, before *Striga* emergence could be part of such an integrated strategy. Reducing *Striga* seed bank in the filed could be an important step to be involved in ISM. Stimulating seed germination of parasitic weeds with the use of a synthetic germination stimulant, in the absence of a host commonly referred to as the 'suicidal germination approach' (Ueno et al. 2011a). The use of strigol analogues as suicidal germinating agents was tested (Mangnus and Zwanenburg 1992; Zwanenburg et al. 2009; Ueno et al. 2011a, b) in the past. Application of GR 24 and GR 7 to *Striga*-infested soils showed up to 50% reduction in the seed population (Mwakaboko and Zwanenburg 2011 a, b). Mwakaboko and Zwanenburg (2011a, b) recently demonstrated about new synthetic readily accessible stimulant molecules and working model for designing new bioactive strigolactones analogues. The use of strigolactone analogue such as Nijmegen-1 reduced the soil seed bank of *Orobanche* by an average of 35 percent (Zwanenburg et al. 2009).

Similarly some calculations were made to see how much reduction of *Striga* seed germination could be obtained if a reduction in germination due to resistance (Res), fertilizer (Fer) or herbicidal (carotenoid inhibitor) (Her) approaches from the present thesis studies are combined (Fig. 1).With regard to fertilizer application the reduction in *Striga* germination was calculated by comparing the germination caused by root exudates of 50%P with the control treatment (0%P). For the carotenoid inhibitor approach the germination induced by plants treated with 0.01 µM fluridone was compared with the control treatment. For the resistance approach the germination induced by an intermediate

rice cv Kairyo-HM was compared with control cv IAC 165. In addition to this, some additive effects by combining the reduction of these strategies were estimated. The individual reduction by using a resistant cultivar, fertilizer and herbicide application were estimated at 62, 72 and 51%, respectively. However, combination of these approaches would be more effective, if we assume that the effects are additive, i.e. the factor has the same relative effect when applied in combination with another factor. For example resistance + fertilizer would theoretically reduce *Striga* germination by 89%. Resistance along with herbicide would result in a reduction of *Striga* germination by up to 81% and fertilizer in combination with herbicide 86%. If all three factors are combined this could theoretically result in a reduction of *Striga* germination of 95%. In *Striga* numbers: if we assume 50 *Striga* seeds germinate in the control treatment, then the use of a resistant variety, fertilizer and herbicide would result in 19, 25 and 14 germinated *Striga* seeds, respectively (Fig. 1). The use of a resistant cultivar in combination with fertilizer would lead to a germination of only 5 seeds and the combination of all three strategies would lead to a germination of only 3 *Striga* seeds (Fig. 1). Further study on how effective combinations of these factors are, is however required. The above mentioned strategies can also be further combined with other *Striga* control measures as indicated in Fig. 2 to develop an integrated control technology that could be ideal, cost effective, efficient, affordable, and useful to small-scale farmers. An example of such combination of control measures/traits is the development of durable resistant cultivars by combining pre- and post-attachment resistance (Cissoko et al. 2011; Jamil et al. 2011c) assuming that varietal control of *Striga* will be an important component in integrated *Striga* management (Rodenburg et al. 2005; Scholes and Press 2008). Post-attachment resistance can complement partial resistance due to low strigolactone production and enhance durability of the resistance, due to the multigenic nature of the combined resistance mechanisms. The combination of such more durable crop resistance with cultural control measures such as the use of fertilizer would further increase the effectiveness. Such an integrated *Striga* management protocol, consisting of a combination of multiple strategies (Fig. 2) could in the end possibly lead to the durable eradication of this noxious weed in cereal production systems in the African continent.

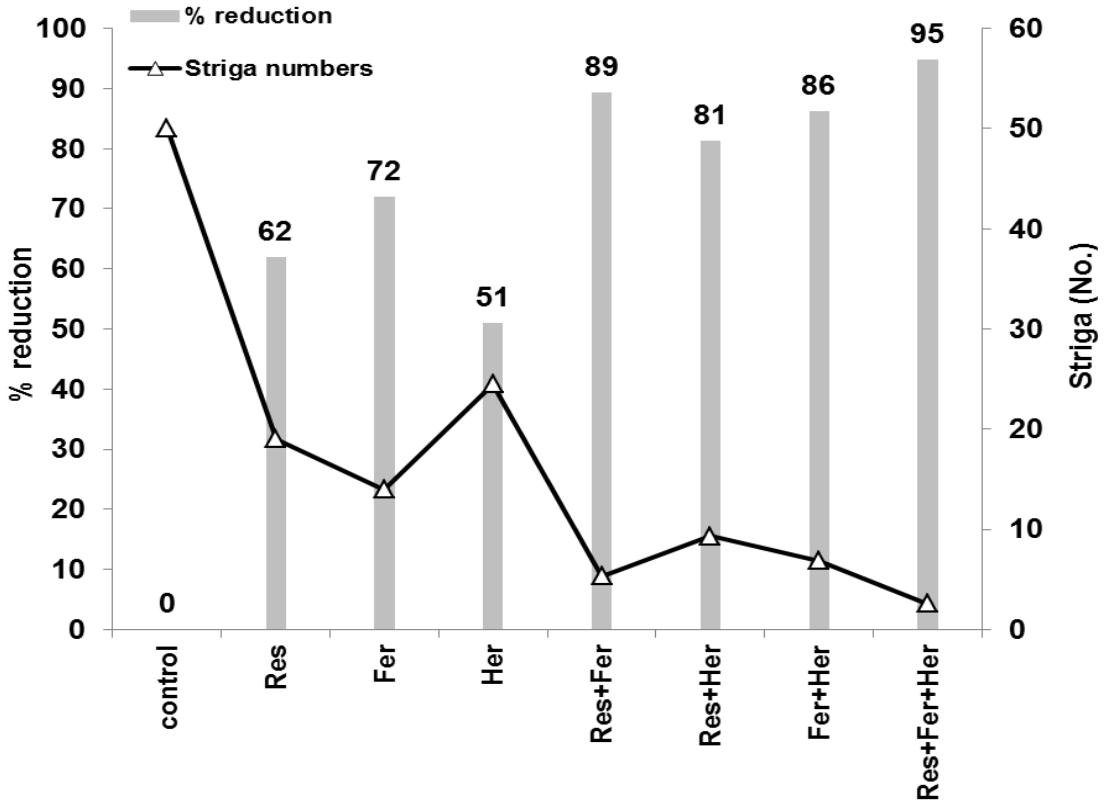


Fig. 1 Individual as well as additive effects of resistance (Res), fertilizers (Fer) and herbicidal (Her) strategies on reduction of *Striga* seed germination

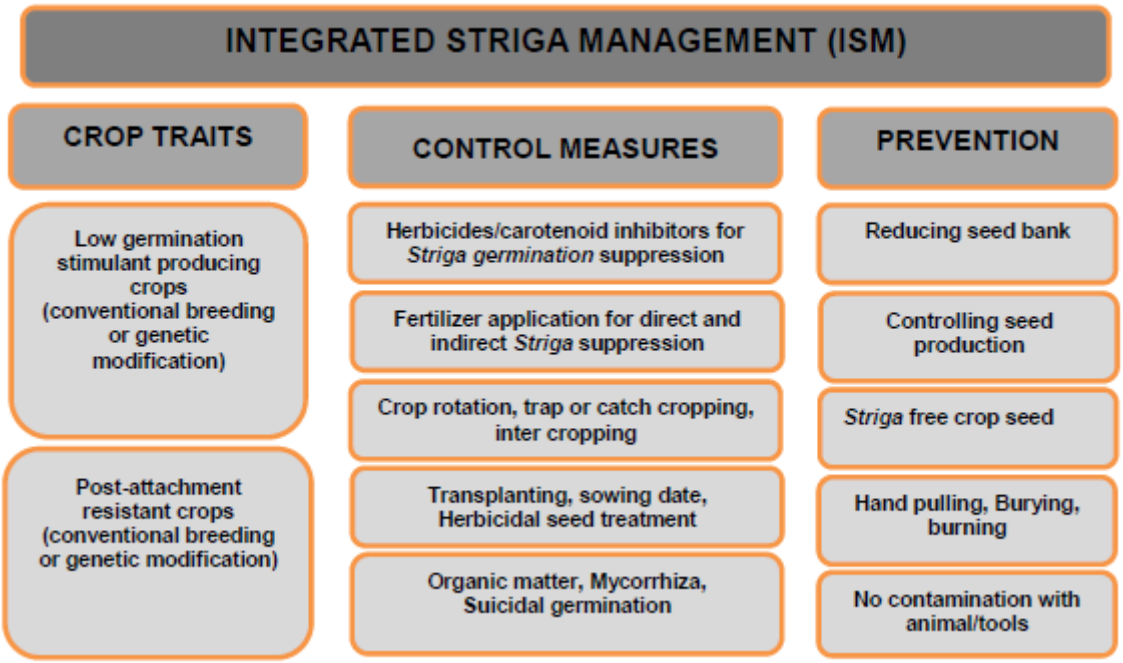


Fig. 2 List of crop traits and control and preventive measures that could result in Integrated *Striga* Management (ISM)

Conclusions

In conclusion, control strategies applied prior to emergence, i.e. targeting germination, could be an effective and efficient strategy against *Striga*. The presence of genetic variation in strigolactone production (and composition) that we report allows for selection of low strigolactone producing cultivars that can exhibit pre-attachment resistance, a cost effective element in developing a *Striga* control strategy. The use of carotenoid biosynthesis inhibitors also showed a fair reduction in strigolactone production and *Striga* infection in cereals, but more studies are needed before this strategy can be used in a practical field application. Improving soil fertility with fertilizers showed a strong inhibiting effect on *Striga* infection due to the reduced production and secretion of strigolactones and consequently lower induction of *Striga* seed germination. Further investigation of the use of pre-attachment resistance, carotenoid inhibitors and fertilizers – alone or in combination with each other and/or other control strategies - under field conditions is needed to optimize these strategies for poor small scale farmers in Africa.

References

Abunyewa AA, Padi FK (2003) Changes in soil fertility and *Striga hermonthica* prevalence associated with legume and cereal cultivation in the Sudan savannah zone of Ghana. Land Degradation & Development 14: 335-343

Ackroyd RD, Graves JD (1997) The regulation of the water potential gradient in the host and parasite relationship between *Sorghum bicolor* and *Striga hermonthica*. Annals of Botany 80: 649-656

Adagba MA, Lagoke STO, Imolehin ED (2002) Nitrogen effect on the incidence of *Striga hermonthica* (Del.) Benth in upland rice. Acta Agronomica Hungarica 50: 145-150

Adetimirin VO, Aken'ova ME, Kim SK (2000) Effects of *Striga hermonthica* on yield components in maize. Journal of Agricultural Science 135: 185-191

Aflakpui GKS, Gregory PJ, Froud-Williams RJ (1998) Effect of temperature on seed germination rate of *Striga hermonthica* (Del.) Benth. Crop Protection 17: 129-133

Africa RC (2007) Africa Rice Trends: Overview of recent developments in the Sub saharan Africa rice sector. Africa Rice Center, Cotonou, Benin.

Ahonsi MO, Berner DK, Emechebe AM, Lagoke ST, Sanginga N (2003) Potential of ethylene-producing pseudomonads in combination with effective N-2-fixing bradyrhizobial strains as supplements to legume rotation for *Striga hermonthica* control. Biological Control 28: 1-10

Akaike H (1981) Citation classic - a new look at the statistical model identification. Current Contents/Engineering Technology & Applied Sciences: 22-22

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435: 824-827

Akiyama K, Hayashi H (2006) Strigolactones: Chemical signals for fungal symbionts and parasitic weeds in plant roots. Annals of Botany 97: 925-931

Akiyama K, Ogasawara S, Ito S, Hayashi H (2010) Structural requirements of strigolactones for hyphal branching in AM Fungi. Plant and Cell Physiology 51: 1104-1117

Anonymous (2007) Africa Rice Trends: Overview of recent developments in the Sub-Saharan Africa rice sector. Africa Rice Center, Cotonou, Benin

Aune JB, Doumbia M, Berthe A (2007) Microfertilizing sorghum and pearl millet in Mali - Agronomic, economic and social feasibility. Outlook on Agriculture 36: 199-203

Awad AA, Sato D, Kusumoto D, Kamioka H, Takeuchi Y, Yoneyama K (2006) Characterization of strigolactones, germination stimulants for the root parasitic plants *Striga* and *Orobanche*, produced by maize, millet and sorghum. Plant Growth Regulation 48: 221-227

Ayongwa GC, Stomph TJ, Emechebe AM, Kuyper TW (2006) Root nitrogen concentration of sorghum above 2% produces least *Striga hermonthica* seed stimulation. Annals of Applied Biology 149: 255-262

Ayongwa GC, Stomph TJ, Hoevers R, Ngoumou TN, Kuyper TW (2010) *Striga* infestation in Northern Cameroon: Magnitude, dynamics and implications for management. Njas-Wageningen Journal of Life Sciences 57: 159-165

Bagayoko M, Buerkert A, Lung G, Bationo A, Romheld V (2000) Cereal/legume rotation effects on cereal growth in Sudano-Sahelian West Africa: soil mineral nitrogen, mycorrhizae and nematodes. Plant and Soil 218: 103-116

Bagayoko M, Maman N, Palé S, Sirifi S, Taonda SJB, Traore S, Mason SC (2011) Microdose and N and P fertilizer application rates for pearl millet in West Africa. African Journal of Agricultural Research 6: 1141-1150

Balasubramanian V, Sie M, Hijmans RJ, Otsuka K (2007) Increasing rice production in Sub-Saharan Africa: Challenges and opportunities. Advances in Agronomy 94: 55-133

Banks PA, Merkle MG (1979) Soil detection and mobility of fluridone. Weed Science 27: 309-312

Bationo A, Ndunguru BJ, Ntare BR, Christianson CB, Mokwunye AU (1991) Fertilizer management strategies for legume based cropping systems in the West African semiarid tropics. Phosphorus nutrition of grain legumes in the semi-arid tropics, pp 213-226

Bebawi FF, Abdelaziz AH (1983) Effects of cultivar mixtures, fertilizer, and plant density on grain sorghum (*Sorghum bicolor*) *Striga hermonthica* relations. Weed Science 31: 552-556

Bekunda MA, Bationo A, Ssali H (1997) Soil fertility management in Africa: A review of selected research trials. Soil Sci. Soc. Amer., Madison, W.I.

Berard DF, Rainey DP, Lin CC (1978) Absorption, translocation, and metabolism of fluridone in selected crop species. Weed Science 26: 252-254

Berner DK, Kling JG, Singh BB (1995) *Striga* research and control - a perspective from Africa. Plant Disease 79: 652-660

Berner D, Carsky R, Dashiell K, Kling J, Manyong V (1996) A land management based approach to integrated *Striga hermonthica* control in sub-Saharan Africa. Outlook on Agriculture 25: 157-164

Boger P (1996) Mode of action of herbicides affecting carotenogenesis. Journal of Pesticide Science 21: 473-478

Böger P, Sandmann G (1998) Carotenoid biosynthesis inhibitor herbicides-mode of action and resistance mechanisms. Pesticide Outlook 9: 29–35

Booker J, Chatfield S, Leyser O (2003) Auxin acts in xylem associated or medullary cells to mediate apical dominance. Plant Cell 15: 495-507

Boukar I, Hess DE, Payne WA (1996) Dynamics of moisture, nitrogen, and *Striga* infestation on pearl millet transpiration and growth. Agronomy Journal 88: 545-549

Bouwmeester HJ, Matusova R, Sun ZK, Beale MH (2003) Secondary metabolite signalling in host-parasitic plant interactions. Current Opinion in Plant Biology 6: 358-364

Bouwmeester HJ, Roux C, Lopez-Raez JA, Becard G (2007) Rhizosphere communication of plants, parasitic plants and AM fungi. Trends in Plant Science 12: 224-230

Breitenbach J, Zhu CF, Sandmann G (2001) Bleaching herbicide norflurazon inhibits phytoene desaturase by competition with the cofactors. Journal of Agricultural and Food Chemistry 49: 5270-5272

Brown GD (1993) Annulide, a sesquiterpene lactone from *Artemisia annua*. Phytochemistry 32: 391-393

Buee M, Rossignol M, Jauneau A, Ranjeva R, Becard G (2000) The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Molecular Plant-Microbe Interactions 13: 693-698

Butler LG (1995) Chemical communication between the parasitic weed *Striga* and its crop host. A new dimension in allelochemistry. In KInderjit FA, Einhellig (eds). Insights into Allelopathy. ACS Symposium Series. ACS Books, Washington, DC. pp 158-168

Cardwell KF, Lane JA (1995) Effect of Soils, cropping system and host phenotype on incidence and severity of *Striga* gesnerioides on cowpea in West Africa. Agriculture Ecosystems & Environment 53: 253-262

Carsky RJ, Singh L, Ndikawa R (1994) Suppression of *Striga hermonthica* on sorghum using a cowpea intercrop. Experimental Agriculture 30: 349-358

Cechin I, Press MC (1993) Nitrogen relations of the sorghum *Striga hermonthica* host parasite association - Growth and photosynthesis. Plant Cell and Environment 16: 237-247

CIMMYT (1988) From agronomic data to farmer recommendations: An economics training manual. Mexico, DF.

Cissoko M, Boisnard A, Rodenburg J, Press M, Scholes JD (2011) New Rice for Africa (NERICA) cultivars exhibit different levels of post-attachment resistance against the parasitic weeds *Striga hermonthica* and *Striga asiatica*. New Phytologist DOI: 10.1111/j.1469-8137.2011.03846.x

Colombia B (2004) Amitrole pesticide info. Ministry of food, agriculture and fisheries: 1-2

Cook CE, Coggon P, McPhail AT, Wall ME, Whichard LP, Egley GH, Luhan PA (1972) Germination stimulants .2. Structure of strigol - potent seed germination stimulant for Witchweed (*Striga* Lutea Lour). Journal of the American Chemical Society 94: 6198-&

Cook CE, Whichard LP, Turner B, Wall ME (1966) Germination of Witchweed (*Striga Lutea* Lour.): Isolation and properties of a potent stimulant. Science 154: 1189-1190.

Crawford S, Shinohara N, Sieberer T, Williamson L, George G, Hepworth J, Muller D, Domagalska MA, Leyser O (2010) Strigolactones enhance competition between shoot branches by dampening auxin transport. Development 137: 2905-2913

Dawoud DA, Sauerborn J (1994) Impact of drought stress and temperature on the parasitic weeds *Striga hermonthica and Alectra vogelii* in their early growth stages. Experimental Agriculture 30: 249-257

De Groot H (2007) *Striga* economics. In: Ejeta G, Gressel J (eds.) Integrating new Technologies for *Striga* Control. World Scientific Co. Ltd. London UK, pp. 265-280.

Dembele I, Kone D, Soumare A, Coulibaly D, Kone Y, Ly B, Kater L (2000) Fallows and field systems in dryland Mali. IIED London

Demmigadams B, Adams WW (1992) Photoprotection and other responses of plants to high light stress. Annual Review of Plant Physiology and Plant Molecular Biology 43: 599-626

Dingkuhn M, Jones MP, Johnson DE, Sow A (1998) Growth and yield potential of *Oryza sativa* and *O. glaberrima* upland rice cultivars and their interspecific progenies. Field Crops Research 57: 57-69

Dun EA, Ferguson BJ, Beveridge CA (2006) Apical dominance and shoot branching. Divergent opinions or divergent mechanisms? Plant Physiology 142: 812-819

Ejeta G, Butler LG, Babiker AGT (1992) New approaches to the control of *Striga*. *Striga* research at Purdue University. Bulletin RB-991 Agricultural Experimental Research Station. West Lafayete, Indiana, Purdue University, USA pp 27

Ejeta G, Mohammed A, Rich P, Melake-Berhan A, Housley TL, Hess DE (2000) Selection for specific mechanisms of resistance to *Striga* in sorghum. Breeding for Striga Resistance in Cereals. Proc. of a workshop held at IITA., Ibadan, Nigeria. Margraf Weikersheim, Germany, pp 29-37

Ejeta G (2005) Integrating biotechnology, breeding, and agronomy in the control of the parasitic weed *Striga* spp in sorghum. In: Tuberosa R (ed) Wake of the Double Helix: From the Green Revolution to the Gene Revolution. Avenue Media, Bologna, Italy, Bologna, Italy, pp 239– 251

Ejeta G (2007) Breeding for *Striga* resistance in sorghum: Exploitation of an intricate host-parasite biology. Crop Science 47: S216-S227

Ekeleme F, Kamara AY, Omoigui LO, Chikoye D, Dugje IY, Tegbaru A (2011) Effect of sowing date on *Striga* infestation and yield of sorghum (*Sorghum bicolor* [L.] Moench) cultivars in the Sudan savanna of northeast Nigeria. African Journal of Agricultural Research 6: 3240-3246

Emechebe AM, Ellis-Jones J, Schulz S, Chikoye D, Douthwaite B, Kureh I, Tarawali G, Hussaini MA, Kormawa P (2004) Farmers' perception of the *Striga* problem and its control in Northern Nigeria. Experimental Agriculture 40: 215-232

Eplee RE, Norris R (1995) Control of parasitic weeds. Chapman & Hall, London

Estabrook EM, Yoder JI (1998) Plant-plant communications: Rhizosphere signaling between parasitic angiosperms and their hosts. Plant Physiology 116: 1-7

Ezeaku IE, Gupta SC (2004) Development of sorghum populations for resistance to *Striga hermonthica* in the Nigerian Sudan Savanna. African Journal of Biotechnology 3: 324-329

FAO (2009) statistical databases. http://faostat.fao.org.

FAO (2009a) Rice market monitor Food and Agriculture Organization of the United Nations Rome, Italy.

Farina MPW, Thomas PEL, Channon P (1985) Nitrogen, phosphorus and potassium effects on the incidence of *Striga asiatica* (L) Kuntze in Maize. Weed Research 25: 443-447

Frost HM (1994) *Striga* research and survey in Kenya. National Agricultural Research Project, KARI/ODA Crop Protection Project, pp 61

Gacheru E, Rao MR (2001) Managing *Striga* infestation on maize using organic and inorganic nutrient sources in western Kenya. International Journal of Pest Management 47: 233-239

Garba A, Fagam AS, Fushison GG (2007) Contribution of environmental factors to tillering and yield of rice during dry season in Bauchi, Nigeria. International Journal of Tropical Agriculture and Food Systems 1: 42-47

Garcia-Garrido JM, Lendzemo V, Castellanos-Morales V, Steinkellner S, Vierheilig H (2009) Strigolactones, signals for parasitic plants and arbuscular mycorrhizal fungi. Mycorrhiza 19: 449-459

Gbehounou G, Pieterse AH, Verkleij JAC (1996) The decrease in seed germination of *Striga hermonthica* in Benin in the course of the rainy season is due to a dying-off process. Experientia 52: 264-267

Gbehounou G, Adango E (2003) Trap crops of *Striga hermonthica*: in vitro identification and effectiveness *in situ*. Crop Protection 22: 395-404

Gbehounou G, Adango E, Hinvi JC, Nonfon R (2004) Sowing date or transplanting as components for integrated *Striga hermonthica* control in grain cereal crops? Crop Protection 23: 379-386

Gebremedhin W, Goudriaan J, Naber H (2000) Morphological, phenological and water use dynamics of sorghum varieties (*Sorghum bicolor*) under *Striga hermonthica* infestation. Crop Protection 19: 61-68

Gethi JG, Smith ME, Mitchell SE, Kresovich S (2005) Genetic diversity of *Striga hermonthica* and *Striga asiatica* populations in Kenya. Weed Research 45: 64-73

Gitsopoulos TK, Froud-Williams RJ (2004) Effects of oxadiargyl on direct-seeded rice and *Echinochloa crus-galli* under aerobic and anaerobic conditions. Weed Research 44: 329-334

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Becard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 455: 189-194

Graves JD, Press MC, Stewart GR (1989) A carbon balance model of the sorghum *Striga hermonthica* host parasite association. Plant Cell and Environment 12: 101-107

Graves JD, Wylde A, Press MC, Stewart GR (1990) Growth and carbon allocation in *Pennisetum typhoides* infected with the parasitic angiosperm *Striga hermonthica*. Plant Cell and Environment 13: 367-373

Gressel J, Hanafi A, Head G, Marasas W, Obilana B, Ochanda J, Souissi T, Tzotzos G (2004) Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions. Crop Protection 23: 661-689

Guo C, Cui W, Feng X, Zhao J, Lu G (2011) Sorghum insect problems and management. Journal of Integrative Plant Biology 53: 178-192

Gurney AL, Press MC, Ransom JK (1995) The parasitic angiosperm *Striga hermonthica* can reduce photosynthesis of its sorghum and maize hosts in the field. Journal of Experimental Botany 46: 1817-1823

Gurney AL, Slate J, Press MC, Scholes JD (2006) A novel form of resistance in rice to the angiosperm parasite *Striga hermonthica*. New Phytologist 169: 199-208

Gworgwor NA, Weber HC (1991) Effect of N application on sorghum growth, *Striga* infestation and the osmotic pressure of the parasite in relation to the host. Journal of Plant Physiology 139: 194-198

Haefele SM, Johnson DE, Diallo S, Wopereis MCS, Janin I (2000) Improved soil fertility and weed management is profitable for irrigated rice farmers in Sahelian West Africa. Field Crops Research 66: 101-113

Harahap Z, Ampongnyarko K, Olela JC (1993) *Striga hermonthica* resistance in upland rice. Crop Protection 12: 229-231

Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annual Review of Microbiology 59: 19-42

Hauck C, Muller S, Schildknecht H (1992) A germination stimulant for parasitic flowering plants from *Sorghum bicolor*, a genuine host plant. Journal of Plant Physiology 139: 474-478

Haussmann BIG, Obilana AB, Blum A, Ayiecho PO, Schipprack W, Geiger HH (1998) Hybrid performance of sorghum and its relationship to morphological and physiological traits under variable drought stress in Kenya. Plant Breeding 117: 223-229

Haussmann BIG, Hess DE, Welz HG, Geiger HH (2000) Improved methodologies for breeding *Striga* resistant sorghums. Field Crops Research 66: 195-211

Hayward A, Stirnberg P, Beveridge C, Leyser O (2009) Interactions between auxin and strigolactone in shoot branching control. Plant Physiology 151: 400-412

Hess DE, Ejeta G (1992) Inheritance of resistance to *Striga* in sorghum genotype SRN39. Plant Breeding 109: 233-241

Hess DE, Ejeta G, Butler LG (1992) Selecting sorghum genotypes expressing a quantitative biosynthetic trait that confers resistance to *Striga*. Phytochemistry 31: 493-497

Hess DE, Williams JH (1994) Influence of planting date on *Striga* infestation and yield of pearl millet. Phytopathology 84: 1104

Hess FD (2000) Light-dependent herbicides: an overview. Weed Science 48: 160-170

Hirsch AM, Bauer WD, Bird DM, Cullimore J, Tyler B, Yoder JI (2003) Molecular signals and receptors: Controlling rhizosphere interactions between plants and other organisms. Ecology 84: 858-868

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. Agricultural Experiment Station, University of California, Berkeley. Circular.

Humphrey AJ, Beale MH (2006) Strigol: Biogenesis and physiological activity. Phytochemistry 67: 636-640

Jamil M, Charnikhova T, Verstappen F, Bouwmeester H (2010) Carotenoid inhibitors reduce strigolactone production and *Striga hermonthica* infection in rice. Archives of Biochemistry and Biophysics 504: 123-131

Jamil M, Charnikhova T, Cardoso C, Jamil T, Ueno K, Verstappen F, Asami T, Bouwmeester H (2011a) Quantification of the relationship between strigolactones and *Striga hermonthica* in rice under varying levels of nitrogen and phosphorus. Weed Research 51: 373-385.

Jamil M, Charnikhova T, Houshyani B, Ast A, Bouwmeester H (2011b) Genetic variation in strigolactone production and tillering in rice and its effect on *Striga hermonthica* infection. Planta: DOI 10.1007/s00425-00011-01520-y

Jamil M, Rodenburg J, Charnikhova T, Bouwmeester H (2011c) Pre-attachment *Striga hermonthica* resistance of NERICA cultivars based on low strigolactone production. New Phytologist: 192: 964-975

Joel DM, Steffens JC, Matthews DE (1995) Germination of weedy root parasites. Marcel Dekker, Inc. New York.

Joel DM (2000) The long term approach to parasitic weeds control: manipulation of specific developmental mechanisms of the parasite. Crop Protection 19: 753-758

Johnson DE, Riches CR, Diallo R, Jones MJ (1997) *Striga* on rice in West Africa; Crop host range and the potential of host resistance. Crop Protection 16: 153-157

Johnson DE, Wopereis MCS, Mbodj D, Diallo S, Powers S, Haefele SM (2004) Timing of weed management and yield losses due to weeds in irrigated rice in the Sahel. Field Crops Research 85: 31-42

Jones MP, Dingkuhn M, Aluko GK, Semon M (1997a) Interspecific *Oryza sativa* L X *O. glaberrima* Steud progenies in upland rice improvement. Euphytica 94: 237-246

Jones MP, Mande S, Aluko K (1997b) Diversity and potential of *Oryza glaberrima* Steud in upland rice breeding. Breeding Science 47: 395-398

Kamara AY, Menkir A, Chikoye D, Omoigui LO, Ekeleme F (2007) Cultivar and nitrogen fertilization effects on *Striga* infestation and grain yield of early maturing tropical maize. Maydica 52: 415-423

Kamara AY, Ellis-Jones J, Amaza P, Omoigui LO, Helsen J, Dugje IY, Kamai N, Menkir A, White RW (2008) A participatory approach to increasing productivity of maize through *Striga hermonthica* control in northeast Nigeria. Experimental Agriculture 44: 349-364

Kanampiu FK, Kabambe V, Massawe C, Jasi L, Friesen D, Ransom JK, Gressel J (2003) Multi-site, multi-season field tests demonstrate that herbicide seed-coating herbicide-resistance maize controls *Striga* spp. and increases yields in several African countries. Crop Protection 22: 697-706

Kapulnik Y, Delaux PM, Resnick N, Mayzlish-Gati E, Wininger S, Bhattacharya C, Sejalon-Delmas N, Combier JP, Becard G, Belausov E, Beeckman T, Dor E, Hershenhorn J, Koltai H (2011a) Strigolactones affect lateral root formation and root-hair elongation in Arabidopsis. Planta 233: 209-216

Kapulnik Y, Resnick N, Mayzlish-Gati E, Kaplan Y, Wininger S, Hershenhorn J, Koltai H (2011b) Strigolactones interact with ethylene and auxin in regulating root-hair elongation in Arabidopsis. Journal of Experimental Botany 62: 2915-2924

Khan ZR, Hassanali A, Overholt W, Khamis TM, Hooper AM, Pickett JA, Wadhams LJ, Woodcock CM (2002) Control of witchweed *Striga hermonthica* by intercropping with Desmodium spp., and the mechanism defined as allelopathic. Journal of Chemical Ecology 28: 1871-1885

Khan ZR, Midega CAO, Hassanali A, Pickett JA, Wadhams LJ, Wanjoya A (2006a) Management of witchweed, *Striga hermonthica*, and stemborers in sorghum, *Sorghum bicolor*, through intercropping with greenleaf desmodium, Desmodium intortum. International Journal of Pest Management 52: 297-302

Khan ZR, Pickett JA, Wadhams LJ, Hassanali A, Midega CAO (2006b) Combined control of *Striga hermonthica* and stemborers by maize-Desmodium spp. intercrops. Crop Protection 25: 989-995

Khan ZR, Midega CAO, Njuguna EM, Arnudavi DM, Wanyama JM, Pickett JA (2008) Economic performance of the 'push-pull' technology for stemborer and *Striga* control in smallholder farming systems in western Kenya. Crop Protection 27: 1084-1097

Kim SK, Adetimirin VO (1997) Responses of tolerant and susceptible maize varieties to timing and rate of nitrogen under *Striga hermonthica* infestation. Agronomy Journal 89: 38-44

Kim SK, Adetimirin VO, Akintunde AY (1997a) Nitrogen effects on *Striga hermonthica* infestation, grain yield, and agronomic traits of tolerant and susceptible maize hybrids. Crop Science 37: 711-716

Kim SK, Lagoke STO, The C (1997b) Observations on field infection by witchweed (*Striga* species) on maize in West and Central Africa. International Journal of Pest Management 43: 113-121

Kim SK, Fajemisin JM, The C, Adepoju A, Kling J, Badu-Apraku B, Versteeg M, Carsky R, Lagoke STO (1998) Development of synthetic maize populations for resistance to *Striga hermonthica*. Plant Breeding 117: 203-209

Kim HK, Luquet D, van Oosterom E, Dingkuhn M, Hammer G (2010a) Regulation of tillering in sorghum: genotypic effects. Annals of Botany 106: 69-78

Kim HK, van Oosterom E, Dingkuhn M, Luquet D, Hammer G (2010b) Regulation of tillering in sorghum: environmental effects. Annals of Botany 106: 57-67

Kohlen W, Charnikhova T, Liu Q, Bours R, Domagalska MA, Beguerie S, Verstappen F, Leyser HMO, Bouwmeester HJ, Ruyter-spira C (2011a) Strigolactones are transported through the xylem and play a key role in shoot architectural response to phosphate deficiency in non-AM host *Arabidopsis thaliana*. Plant Physiology 155: 974-987

Kohlen W, López Ráez JA, Pollina T, Lammers M, Toth P, Charnikhova T, Maagd R, Pozo MJ, Bouwmeester H, Ruyter-Spira C (2011b) The tomato CAROTENOID CLEAVAGE DIOXYGENASE8 (SlCCD8) is regulating, rhizosphere signaling, plant architecture and reproductive development through strigolactone biosynthesis. The Plant Journal (Submitted)

Kohlen W, Ruyter-Spira C, Bouwmeester HJb (2011c) Strigolactones: A new musician in the orchestra of plant hormones. Botany 89: 827-840

Koltai H, Dor E, Hershenhorn J, Joel DM, Weininger S, Lekalla S, Shealtiel H, Bhattacharya C, Eliahu E, Resnick N, Barg R, Kapulnik Y (2010) Strigolactones effect on root growth and root hair elongation may be mediated by auxin efflux carriers. Journal of Plant Growth Regulation 29: 129-136

Labrousse P, Arnaud MC, Serieys H, Berville A, Thalouarn P (2001) Several mechanisms are involved in resistance of *Helianthus* to *Orobanche cumana* Wallr. Annals of Botany 88: 859-868

Lagoke STO, Parkinson V, Agunbiade RM (1991) Parasitic weeds and control methods in Africa. Proceedings Combating Striga in Africa. IITA, Ibadan, Nigeria, pp 3-14

Lajtha K, Harrison AF (1995) Strategies of phosphorus acquisition and conservation by plant species and communities. John Wiley Sons Ltd, Chichester, UK

Leyser O (2003) Regulation of shoot branching by auxin. Trends in Plant Science 8: 541-545

Leyser O (2008) Strigolactones and shoot branching: A new trick for a young dog. Developmental Cell 15: 337-338

Lin H, Wang R, Qian Q, Yan M, Meng X, Fu Z, Yan C, Jiang B, Su Z, Li J, Wang Y (2009) DWARF27, an iron containing protein required for the biosynthesis of strigolactones, regulatesrice tiller bud outgrowth. Plant Cell 21: 1512-1525

Ljung K, Bhalerao RP, Sandberg G (2001) Sites and homeostatic control of auxin biosynthesis in Arabidopsis during vegetative growth. Plant Journal 28: 465-474

Logan DC, Stewart GR (1992) Germination of the seeds of parasitic angiosperms. Seed Science Research 2: 179-190

Lopez-Raez JA, Bouwmeester H (2008) Fine-tuning regulation of strigolactone biosynthesis under phosphate starvation. Plant Signal Behav 3: 963-965

Lopez-Raez JA, Charnikhova T, Gomez-Roldan V, Matusova R, Kohlen W, De Vos R, Verstappen F, Puech-Pages V, Becard G, Mulder P, Bouwmeester H (2008) Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. New Phytologist 178: 863-874

Lopez-Raez JA, Matusova R, Cardoso C, Jamil M, Charnikhova T, Kohlen W, Ruyter-Spira C, Verstappen F, Bouwmeester H (2009) Strigolactones: ecological significance and use as a target for parasitic plant control. Pest Management Science 65: 471-477

Lynn DG, Chang M (1990) Phenolic signals in cohabitation - implications for plant development. Annual Review of Plant Physiology and Plant Molecular Biology 41: 497-526

Mangnus EM, Zwanenburg B (1992) Tentative molecular mechanism for germination stimulation of *Striga* and *Orobanche* seeds by strigol and its synthetic analogues. Journal of Agric. Food Chem 40: 1066-1070

Maiti RK, Ramaiah KV, Bisen SS, Chidley VL (1984) A comparative study of the haustorial development of *Striga asiatica* (L.) Kuntze on Sorghum cultivars. Annals of Botany 54: 447-457

Matusova R, van Mourik T, Bouwmeester HJ (2004) Changes in the sensitivity of parasitic weed seeds to germination stimulants. Seed Science Research 14: 335-344

Matusova R, Rani K, Verstappen FWA, Franssen MCR, Beale MH, Bouwmeester HJ (2005) The strigolactone germination stimulants of the plant-parasitic *Striga* and Orobanche spp. are derived from the carotenoid pathway. Plant Physiology 139: 920-934

Matusova R, Bouwmeester HJ (2006) The effect of host root derived chemical signals on the germination of parasitic plants. Springer, Wageningen

Mersie W, Singh M (1987) Comparison of norflurazon absorption by excised roots of 3 plant-species Pesticide Biochemistry and Physiology 28: 114-120

Mohamed AH, Housley TL, Ejeta G (2010) An in vitro technique for studying specific *Striga* resistance mechanisms in sorghum. African Journal of Agricultural Research 5: 1868-1875

Mori K, Matsui J, Yokota T, Sakai H, Bando M, Takeuchi Y (1999) Structure and synthesis of orobanchol, the germination stimulant for *Orobanche minor*. Tetrahedron Letters 40: 943-946

Muller S, Hauck C, Schildknecht H (1992) Germination stimulants produced by *Vigna unguiculata* Walp *cv* Saunders upright. Journal of Plant Growth Regulation 11: 77-84

Musselman LJ (1980) The biology of *Striga*, Orobanche, and other root parasitic weeds. Annual Review of Phytopathology 18: 463-489

Mwakaboko AS, Zwanenburg B (2011a) Strigolactone analogs derived from ketones using a working model for germination stimulants as a blueprint. Plant and Cell Physiology 52: 699-715

Mwakaboko AS, Zwanenburg B (2011b) Single step synthesis of strigolactone analogues from cyclic keto enols, germination stimulants for seeds of parasitic weeds. Bioorganic & Medicinal Chemistry 19: 5006-5011

Nagahashi G, Douds DD (2000) Partial separation of root exudate components and their effects upon the growth of germinated spores of AM fungi. Mycological Research 104: 1453-1464

Netzly DH, Riopel JL, Ejeta G, Butler LG (1988) Germination stimulants of witchweed (*Striga asiatica*) from hydrophobic root exudate of Sorghum (*Sorghum bolor*). Weed Science 36: 441-446

Nickrent DL, Musselman LJ (2004) Introduction to parasitic flowering plants. The Plant Health Instructor. DOI: 10.1094/PHI-I-2004-0330-01

Olivier A, Benhamou N, Leroux GD (1991) Cell surface Interactions between sorghum roots and the parasitic weed *Striga hermonthica*- Cytochemical aspects of cellulose distribution in resistant and susceptible host tissues. Canadian Journal of Botany-Revue Canadienne De Botanique 69: 1679-1690

Ongaro V, Leyser O (2007) Shoot branching in Arabidopsis. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 146: S237-S238

Oryokot J (1994) *Striga*: Strategies for its control. African Crop Science Conference Proceedings 1: 224-226

Oswald A, Ransom JK (2001) *Striga* control and improved farm productivity using crop rotation. Crop Protection 20: 113-120

Oswald A, Ransom JK (2004) Response of maize varieties to *Striga* infestation. Crop Protection 23: 89-94

Oswald A (2005) *Striga* control - technologies and their dissemination. Crop Protection 24: 333-342

Oyebande L (2001) Water problems in Africa - how can the sciences help? Hydrological Sciences Journal-Journal Des Sciences Hydrologiques 46: 947-962

Pageau K, Simier P, Naulet N, Robins R, Fer A (1998) Carbon dependency of the hemiparasite *Striga hermonthica* on *Sorghum bicolor* determined by carbon isotopic and gas exchange analyses. Australian Journal of Plant Physiology 25: 695-700

Pageau K, Simier P, Le Bizec B, Robins RJ, Fer A (2003) Characterization of nitrogen relationships between *Sorghum bicolor* and the root-hemiparasitic angiosperm *Striga hermonthica* (Del.) Benth. using (KNO3)-N-15 as isotopic tracer. Journal of Experimental Botany 54: 789-799

Palé S, Mason SC, Taonda SJB (2009) Water and fertilizer influence on yield of grain sorghum varieties produced in Burkina Faso. South Afr. J. Plant Soil 26: 91-97

Parker C (1991) Protection of crops against parasitic weeds. Crop Protection 10: 6-22

Parker C, Riches CR (1993) Parasitic weeds of the world. Oxfordshire: CAB International Wallingford, pp 36-47

Parker C (2009) Observations on the current status of *Orobanche* and *Striga* problems worldwide. Pest Management Science 65**:** 453-459.

Peter GF, Thornber JP (1991) Biochemical composition and organization of higher plant photosystem-ii light harvesting pigment proteins. Journal of Biological Chemistry 266: 16745-16754

Pieterse AH, Verkleij JAC (1991) Effect of soil conditions on *Striga* development – a review. In: J.K. R, Musselman LJ, Worsham AD, Parker C (eds) Proceeding 5th International Symposium on Parasitic Weeds, CIMMYT: Nairobi Kenya., pp 329-339.

Pingali P (2001) World maize facts and trends. Meeting world maize needs: Technological opportunities and priorities for the private sector. CIMMYT, Mexico, D.F., pp 1-21

Pomeranzy Y (1987) Modern cereal science and technology. VCH Publishers, Weinheim

Press MC, Gurney AL (2000) Plant eats plant: sap-feeding witchweeds and other parasitic angiosperms. Biologist (London, England) 47: 189-193

Raju PS, Osman MA, Soman P, Peacock JM (1990) Effects of N, P and K on *Striga asiatica* (L) Kuntze seed germination and infestation of sorghum. Weed Research 30: 139-144

Ramaiah KV, Parker C, Vasudeva, Rao MJ, Musselman LJ (1983) *Striga* identification and control handbook. In: Patancheru AP (ed), India pp 55-64

Ramaiah KV (1987) Breading cereal grains for resistance to witchweed. Academic Press, New York

Ramaiah KV, Chidley VL, House LR (1991) A time course study of early establishment stages of parasitic angiosperm *Striga* asiatica on susceptible sorghum roots. Annals of Applied Biology 118: 403-410

Rani K, Zwanenburg B, Sugimoto Y, Yoneyama K, Bouwmeester HJ (2008) Biosynthetic considerations could assist the structure elucidation of host plant produced rhizosphere signalling compounds (strigolactones) for arbuscular mycorrhizal fungi and parasitic plants. Plant Physiology and Biochemistry 46: 617-626

Ransom JK (2000) Long term approaches for the control of *Striga* in cereals: field management options. Crop Protection 19: 759-763

Reda F, Verkleij JAC, Ernst WHO (2005) Intercropping for the improvement of sorghum yield, soil fertility and *Striga* control in the subsistence agriculture region of Tigray (northern Ethiopia). Journal of Agronomy and Crop Science 191: 10-19

Rich PJ, Grenier U, Ejeta G (2004) *Striga* resistance in the wild relatives of sorghum. Crop Science 44: 2221-2229

Riopel JL, Timko MP (1995) Haustorial initiation and differentiation. Chapman and Hall, London

Rodenburg J, Bastiaans L, Weltzien E, Hess DE (2005) How can field selection for *Striga* resistance and tolerance in sorghum be improved? Field Crops Research 93: 34-50

Rodenburg J, Bastiaans L, Kropff MJ (2006a) Characterization of host tolerance to *Striga hermonthica*. Euphytica 147: 353-365

Rodenburg J, Bastiaans L, Kropff MJ, Van Ast A (2006b) Effects of host plant genotype and seedbank density on *Striga* reproduction. Weed Research 46: 251-263

Rodenburg J, Diagne A, Oikeh S, Futakuchi K, Kormawa PM, Semon M, Akintayo I, Cissé B, Sié M, Narteh L, Nwilene F, Diatta S, Sere Y, Ndjiondjop MN, Youm O, Keya SO (2006c) Achievements and impact of NERICA on sustainable rice production in sub-Saharan Africa. Internal Rice Commission Newsletter 45-58

Rodenburg J, Johnson DE (2009) Weed management in rice based cropping systems in Africa. Advances in Agronomy, Vol 103, pp 149-218

Rodenburg J, Riches CR, Kayeke JM (2010) Addressing current and future problems of parasitic weeds in rice. Crop Protection 29: 210-221

Rodenburg J, Bastiaans L (2011) Host plant defence against *Striga* spp. : reconsidering the role of tolerance. Weed Research 51: 438-441

Roman B, Torres AM, Rubiales D, Cubero JI, Satovic Z (2002) Mapping of quantitative trait loci controlling broomrape (*Orobanche crenata* Forsk.) resistance in faba bean (*Vicia faba* L.). Genome 45: 1057-1063

Ruyter-Spira C, Kohlen W, Charnikhova T, van Zeijl A, van Bezouwen L, de Ruijter N, Cardoso C, Lopez-Raez JA, Matusova R, Bours R, Verstappen F, Bouwmeester H (2011) Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in arabidopsis: another belowground role for strigolactones? Plant Physiology 155: 721-734

Sanchez PA, Shepherd KD, Soule MJ, Place FM, Buresh RJ, Izac AN (1997) Soil fertility replenishment in Africa: An investment in natural resource capital. Soil Scienc Society of America Special Number 51

Sauerborn J, Sprich H, Mercer-Quarshie H (2000) Crop rotation to improve agricultural production in sub-Saharan Africa. Journal of Agronomy and Crop Science-Zeitschrift Fur Acker Und Pflanzenbau 184: 67-72

Sauerborn J, Kranz B, Mercer-Qviarshie H (2003) Organic amendments mitigate heterotrophic weed infestation in savannah agriculture. Applied Soil Ecology 23: 181-186

Schmitz G, Theres K (2005) Shoot and inflorescence branching. Current Opinion in Plant Biology 8: 506-511

Scholes JD, Swarbrick PJ, Slate J, Press MC (2007) The molecular genetic basis of host resistance to *Striga* species; a weay forward. . World Scientific, London, United Kingdom

Scholes JD, Press MC (2008) *Striga* infestation of cereal crops - an unsolved problem in resource limited agriculture. Current Opinion in Plant Biology 11: 180-186

Scott JE, Weston LA, Chappell J, Hanley K (1994) Effects of clomazone on ipp isomerase and prenyl transferase activities in cell-suspension cultures and cotyledons of solanaceous species. Weed Science 42: 509-516

Shah N, Smirnoff N, Stewart GR (1987) Photosynthesis and stomatal characteristics of *Striga hermonthica* in relation to its parasitic habit. Physiologia Plantarum 69: 699-703

Showemimo FA, Kimbeng CA, Alabi SO (2002) Genotypic response of sorghum cultivars to nitrogen fertilization in the control of *Striga hermonthica*. Crop Protection 21: 867-870

Siame BA, Weerasuriya Y, Wood K, Ejeta G, Butler LG (1993) Isolation of Strigol, a germination stimulant for *Striga asiatica*, from host plants. Journal of Agricultural and Food Chemistry 41: 1486-1491

Sieberer T, Leyser O (2006) Plant science auxin transport, but in which direction? Science 312: 858-860

Simier P, Constant S, Degrande D, Moreau C, Robins RJ, Fer A, Thalouarn P (2006) Impact of nitrate supply in C and N assimilation in the parasitic plant *Striga hermonthica* (Del.) Benth (*Scrophulariaceae*) and its host *Sorghum bicolor* L. Plant Cell and Environment 29: 673-681

Smaling EMA, Stein A, Sloot PHM (1991) A statistical analysis of the influence of *Striga hermonthica* on maize yields in fertilizer trials in southwestern Kenya. Plant and Soil 138: 1-8

Smith LH, Keys AJ, Evans MCW (1995) *Striga hermonthica* decreases photosynthesis in Zea mays through effects on leaf cell structure. Journal of Experimental Botany 46: 759-765

Stenersen J (2004) Chemical pesticides: mode of action and toxicology. CRC Press, Florida

Stirnberg P, Ward S, Leyser O (2010) Auxin and strigolactones in shoot branching: intimately connected? Biochemical Society Transactions 38: 717-722

Sun Z, Matusova R, Bouwmeester H (2007) Germination of *Striga* and chemical signaling involved: A target for control methods In: Integrating new technologies for Striga control. World Scientific Publishing Co. Pte. Ltd., London

Sun Z, Hans J, Walter MH, Matusova R, Beekwilder J, Verstappen FWA, Ming Z, van Echtelt E, Strack D, Bisseling T, Bouwmeester HJ (2008) Cloning and characterisation of a maize carotenoid cleavage dioxygenase (ZmCCD1) and its involvement in the biosynthesis of apocarotenoids with various roles in mutualistic and parasitic interactions. Planta 228: 789-801

Tabo R, Bationo A, Gerard B, Ndjeunga J, Marchal D, Amadou B, Annou MG, Sogodogo D, Taonda JBS, Hassane O, Diallo MK, Koala S (2007) Improving cereal productivity and farmers' income using a strategic application of fertilizers in West Africa. Advances in Integrated Soil Fertility Management in Sub-Saharan Africa: Challenges and Opportunities, pp 201-208

Tarfa RA, Kuresh I, Kuchinda NC, Shinggu A, Omolehin RA, Alabi SO, Ado SG (2001) Influence of initial soil physico-chemical properties on *Striga* and maize crop parameters under improved management practices. In: Badu-Apraku B, Fakorede MAB, Ouedraogo M, Carsky RJ, Menkir A (eds) The Proceedings of international workshop on Maize Revolution in Westand Central Africa West and Central Collaborative Maize Research Network, IITA Cotonou, Benin republic pp 303-313

Taylor A, Martin J, Seel WE (1996) Physiology of the parasitic association between maize and witchweed (*Striga hermonthica*): Is ABA involved? Journal of Experimental Botany 47: 1057-1065

ter Braak CJF (1988) Canoco - an extension of decorana to analyze species environment relationships. Vegetatio 75: 159-160

Thomas JM, Steve CW, Floyd MA (2002) Pigment Inhibitors In: Weed Science: Principles and Practices. Wiley-Blackwell New York

Tisdale SL, Nelson WL, Havlin J, Beaton J, Havlin JL, Beaton JD (2004) Soil fertility and fertilizers: an introduction to nutrient management. Prentice Hall, cop, New Jersey

Tittonell P, Vanlauwe B, Leffelaar PA, Giller KE (2005) Estimating yields of tropical maize genotypes from non-destructive, on-farm plant morphological measurements. Agriculture Ecosystems & Environment 105: 213-220

Ueno K, Hanada A, Yamaguchi S, Asami T (2010) Preparation of multideuterated 5-deoxystrigol for use as an internal standard for quantitative LC/MS. Journal of Labelled Compounds and Radiopharmaceuticals DOI: 10.1002/jlcr.1789

Ueno K, Fujiwara M, Nomura S, Mizutani M, Sasaki M, Takikawa H, Sugimoto Y (2011) Structural requirements of strigolactones for germination induction of *Striga gesnerioides* seeds. Journal of Agricultural and Food Chemistry 59: 9226-9231.

Ueno K, Nomura S, Muranaka S, Mizutani M, Takikawa H, Sugimoto Y (2011) Ent-2 '-epi-orobanchol and its acetate, as germination stimulants for *Striga gesnerioides* seeds isolated from cowpea and red clover. Journal of Agricultural and Food Chemistry 59: 10485-10490

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S (2008) Inhibition of shoot branching by new terpenoid plant hormones. Nature 455: 195-U129

Umehara M, Hanada A, Magome H, Takeda-Kamiya N, Yamaguchi S (2010) Contribution of strigolactones to the inhibition of tiller bud outgrowth under phosphate deficiency in rice. Plant Cell Physiol 51: 1118-1126

UNFPA (2008) World population prospects. The 2006 revision and world urbanization prospects: The 2005 revision. http://esa.un.org/unpp.

Vanlauwe B, Kanampiu F, Odhiambo GD, De Groote H, Wadhams LJ, Khan ZR (2008) Integrated management of *Striga hermonthica*, stemborers, and declining soil fertility in western Kenya. Field Crops Research 107: 102-115

Vanlauwe B, Ramisch JJ, Sanginga N (2006) Integrated soil fertility management in Africa: From knowledge to implementation. Biological Approaches to Sustainable Soil Systems 113: 257-272

Verkleij JAC, Kuiper E (2000) Various approaches to controlling root parasitic weeds. Biotechnology and Development Monitor: 16-19

Vogel JT, Walter MH, Giavalisco P, Lytovchenko A, Kohlen W, Charnikhova T, Simkin AJ, Goulet C, Strack D, Bouwmeester H, Fernie AR, Klee HJ (2010) SlCCD7 controls strigolactone biosynthesis, shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. Plant Journal 61: 300-311

Vogler RK, Ejeta G, Butler LG (1996) Inheritance of low production of *Striga germination* stimulant in sorghum. Crop Science 36: 1185-1191

Wang Y, Li J (2011) Branching in rice. Current Opinion in Plant Biology 14: 94-99

Ward SP, Leyser O (2004) Shoot branching. Current Opinion in Plant Biology 7: 73-78

WARDA (2005) Rice trends in sub-Saharan Africa

Watling JR, Press MC (2001) Impacts of infection by parasitic angiosperms on host photosynthesis. Plant Biology 3: 244-250

Weber JB, Shea PH, Weed SB (1986) Fluridone retention and release in soils. Soil Science Society of America Journal 50: 582-588

Weber G, Elemo K, Lagoke STO, Awad A, Oikeh S (1995) Population-dynamics and determinants of *Striga hermonthica* on maize and sorghum in Savanna Farming Systems. Crop Protection 14: 283-290

Weerasuriya Y, Siame BA, Hess D, Ejeta G, Butler LG (1993) Influence of conditions and genotype on the amount of *Striga* germination stimulants exuded by roots of several host crops. Journal of Agricultural and Food Chemistry 41: 1492-1496

WHO (2008) Global and regional food consumption patterns and trends. World Health Organization http://www.who.int/nutrition/topics/3_food consumption/en/index/html

Wolfe AD, Randle CP, Liu L, Steiner KE (2005) Phylogeny and biogeography of *Orobanchaceae*. Folia Geobotanica 40

Wopereis MCS, Diagne A, Rodenburg J, Sié M, Somado EA (2008) Why NERICA is a successful innovation for African farmers: a response to Orr et al. from the Africa Rice Center. Outlook on Agriculture 37: 169-176

Wu GW, Wilson LT, McClung AM (1998) Contribution of rice tillers to dry matter accumulation and yield. Agronomy Journal 90: 317-323

Xie X, Kusumoto D, Takeuchi Y, Yoneyama K, Yamada Y (2007) 2 '-Epi-orobanchol and solanacol, two unique strigolactones, germination stimulants for root parasitic weeds, produced by tobacco. Journal of Agricultural and Food Chemistry 55: 8067-8072

Xie X, Yoneyama K, Kusumoto D, Yamada Y, Takeuchi Y, Sugimoto Y, Yoneyama K (2008) Sorgomol, germination stimulant for root parasitic plants, produced by *Sorghum bicolor*. Tetrahedron Letters 49: 2066-2068

Xie X, Yoneyama K, Harada Y, Fusegi N, Yamada Y, Ito S, Yokota T, Takeuchi Y, Yoneyama K (2009a) Fabacyl acetate, a germination stimulant for root parasitic plants from *Pisum sativum*. Phytochemistry 70: 211-215

Xie X, Yoneyama K, Kurita J-y, Harada Y, Yamada Y, Takeuchi Y, Yoneyama K (2009b) 7-Oxoorobanchyl acetate and 7-oxoorobanchol as germination stimulants for root parasitic plants from flax (*Linum usitatissimum*). Bioscience Biotechnology and Biochemistry 73: 1367-1370

Xie X, Yoneyama K, Yoneyama K (2010) The strigolactone story. Annual Review of Phytopathology 48:93-117

Yoder JI (1999) Parasitic plant responses to host plant signals: a model for subterranean plant-plant interactions. Current Opinion in Plant Biology 2: 65-70

Yoder JI (2001) Host plant recognition by parasitic *Scrophulariaceae*. Current Opinion in Plant Biology 4: 359-365

Yokota T, Sakai H, Okuno K, Yoneyama K, Takeuchi Y (1998) Alectrol and orobanchol, germination stimulants for *Orobanche minor*, from its host red clover. Phytochemistry 49: 1967-1973

Yoneyama K, Takeuchi Y, Yokota T (2001) Production of clover broomrape seed germination stimulants by red clover root requires nitrate but is inhibited by phosphate and ammonium. Physiologia Plantarum 112: 25-30

Yoneyama K, Xie XN, Kusumoto D, Sekimoto H, Sugimoto Y, Takeuchi Y (2007a) Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. Planta 227: 125-132

Yoneyama K, Yoneyama K, Takeuchi Y, Sekimoto H (2007b) Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites. Planta 225: 1031-1038

Yoneyama K, Xie X, Sekimoto H, Takeuchi Y, Ogasawara S, Akiyama K, Hayashi H, Yoneyama K (2008) Strigolactones, host recognition signals for root parasitic plants and arbuscular mycorrhizal fungi, from Fabaceae plants. New Phytologist 179: 484-494

Yoneyama K, Xie X, Takeuchi Y (2009a) Strigolactones: structures and biological activities. Pest Management Science 65: 467-470

Yoneyama K, Xie XN, Takeuchi Y (2009b) Structural diversity and distribution of strigolactones in the plant kingdom. Journal of Pesticide Science 34: 302-305

Yoneyama K, Awad AA, Xie XN, Takeuchi Y (2010) Strigolactones as germination stimulants for root parasitic plants. Plant and Cell Physiology 51: 1095-1103

Yoshida S, Shirasu K (2009) Multiple layers of incompatibility to the parasitic witchweed, *Striga hermonthica*. New Phytologist 183: 180-189

Zimdahl RL (2007) Fundamentals of weed science London Academic Press.

Zwanenburg B, Mwakaboko AS, Reizelman A, Anilkumar G, Sethumadhavan D (2009) Structure and function of natural and synthetic signalling molecules in parasitic weed germination. Pest Management Science 65: 478-491

Summary

Cereal production in Africa is under increasing constraint due to the obligate, out-crossing, hemiparasitic weed *Striga hermonthica* (Del.) Benth, a member of the Scrophulariaceae family. *Striga* parasitizes roots of cereals like sorghum, pearl millet, maize and upland rice. It has infested about 40% of the African agricultural land, resulting in severe yield losses or even complete crop failure worth US$7 billion per annum. The subsistence farmers or approximately 300 million African people lose about 20-80% of their crop because of this weed. This considerable damage by *Striga* is due to the fact that existing control measures are often ineffective. These include cultural and mechanical measures, such as hand pulling, that are mainly adopted after its emergence. Since much of the damage occurs underground during the early stages of parasitism, there is a need to develop control strategies that target the weed prior to attachment and emergence. A crucial step in the lifecycle of *Striga* is the induction of germination by strigolactones, signalling molecules secreted by the roots of its host. These strigolactones could be an important target to control this weed at the pre attachment phase. Control methods targeted at the germination and attachment phase, based on low strigolactones, might prove to be more effective and result in reduced infestation of this weed in cereal crops. In my thesis we studied the relationship between strigolactones and *Striga* infection in cereals and explored opportunities for lowering *Striga* damage at the germination or attachment phase. To this end different aspects like strigolactone biosynthetic inhibitors, genetic variation for strigolactone production, and the effect of fertilizers on strigolactone production were investigated in laboratory studies and – when possible - in the field in Kenya and Mali.

The first investigation was on the use of carotenoid inhibitors to see the possibilities of strigolactone reduction in the roots of plants by blocking carotenoid biosynthesis. We postulated in this study that the (mild) inhibition of carotenoid biosynthesis by carotenoid inhibitors, could lead to a reduced production of strigolactones and decreased *Striga* germination and infection. Very low concentrations of four different carotenoid inhibitors (fluridone, norflurazon, clomazone and amitrole) were applied to rice either through irrigation or through foliar spray. Irrigation application of all carotenoid inhibitors and spray application of amitrole significantly decreased strigolactone production. A significant negative relationship between inhibitor concentration and *Striga* germination and attachment was noted for irrigation application of fluridone, clomazone and norflurazon while amitrole application showed significance only in *Striga* germination. Application of carotenoid inhibitors caused 61-75% reduction in *Striga* germination and 65-94% reduction in *Striga* attachment. The study shows that the reducing effect of carotenoid inhibitors (which, in much higher

concentrations are widely used as herbicides) on strigolactone secretion and subsequent *Striga* germination and attachment may be developed into an attractive *Striga* control technology.

Another experiment (Chapter 3) was aimed at assessing the pre-attachment *Striga* resistance based on low strigolactone production. We hypothesized that low strigolactones producing crop cultivars might possess pre-attachment *Striga* resistance due to less germination. For this purpose a set of 18 upland cultivars of NERICA and their parents were screened for strigolactones production and *Striga* infection parameters like germination, attachment, emergence and *Striga* dry biomass. NERICA 1 and CG14 produced significantly less strigolactones and showed less *Striga* infection while NERICAs 7, 8, 11 and 14 produced the highest amounts of strigolactones and showed the most severe *Striga* infection. A positive relationship between the amount of strigolactones and *Striga* infection was seen among the rice cultivars. This study shows that genetic variation for pre-attachment *Striga* resistance exists in NERICA rice due to variation in strigolactones. This could be highly relevant for breeding programs aimed at the development of *Striga* resistant cultivars.

In Chapter 4 we hypothesized that variation in strigolactone production in rice might be interconnected with the tillering phenotype and that this link could affect *Striga* infection. In this study the genetic variation was tested in a series of rice varieties collected from all over the world for strigolactone production, tillering phenotype and *Striga* infection. Rice cultivars like IAC 165, IAC 1246, Gangweondo and Kinko produced high amounts of the strigolactones, displayed low amounts of tillers and induced high *Striga* germination, attachment, emergence as well as *Striga* biomass. In contrast to this, rice cultivars such as Super Basmati, TN 1, Anakila and Agee showed low production of strigolactones and also low *Striga* germination and infection but high tillering. Statistical analysis across all the varieties confirmed a strong positive correlation between strigolactone production and *Striga* infection and a negative relationship with tillering. These results show that genetic variation in strigolactone production results in variation in tillering and also in *Striga* infection. The tillering phenotype could possibly be used as an easy indicator of the strigolactone production in a breeding programme for *Striga* resistance.

A number of experiments (Chapters 5, 6, 7) were designed with the aim to quantify the relationship between strigolactones and *Striga* germination and attachment and to explore the mechanism responsible for the reported reduction in *Striga* parasitism in the field after fertilizer application. We hypothesized that a better mineral nutrient supply reduces *Striga* infection by reducing strigolactone exudation into the rhizosphere. Different levels of nitrogen and phosphorous were applied under greenhouse conditions using rice, maize and sorghum. For maize and sorghum, a parallel study was carried out under field conditions in Kenya and Mali to study the translation of greenhouse results to the field. Application of N and P effectively suppressed *Striga* infection in the greenhouse in all three crop species and the reduction strongly correlated with reduced secretion of strigolactones into the rhizosphere and the *Striga* germination induced by these exudates. Production

of strigolactones also differed strongly between crop cultivars. Rice *cv* IAC 165 produced about 100-fold higher amounts of 2'-epi-5-deoxystrigol, orobanchol and three new strigolactones than TN 1. Although the field results with maize in Kenya were less consistent than in the greenhouse, especially with respect to P effect, still there was a trend that fertilizer application reduced *Striga* infection. Microdosing of diammonium phosphate fertilizer in sorghum in the field in Mali also showed considerable *Striga* suppression which correlated with the results on strigolactone production and *Striga* infection in the greenhouse. These results show that the positive effect of fertilizer against *Striga* is at least partly due to a reduction in strigolactone production and as a consequence of that lower *Striga* germination and subsequent attachment. However, further research to optimize field application of fertilizers for *Striga* is needed.

Overall it can be concluded that there is a good correlation between strigolactones and *Striga* germination, attachment and biomass. We found this using strigolactone biosynthesis inhibitors, genetic variation and using fertilizer application. These technologies can hence be exploited as an important tool to target *Striga* at a very early phase of its life cycle. The practical field application of these strategies requires further research but could lead to effective *Striga* control components that can be used in Integrated *Striga* Management.

Samenvatting

Graanproductie in Afrika staat onder toenemende druk van parasitaire plant *Striga hermonthica* (Del.) Benth. (*Striga*). Dit lid van de *Scrophulariaceae* familie, parasiteert de wortels van graangewassen zoals sorghum, parelgierst, maïs en hooglandrijst. Ongeveer 40% van alle landbouwgronden in Afrika zijn besmet met het zaad van deze parasiet. Dit leidt tot enorme oogstverliezen welke kunnen oplopen tot zelfs volledige mislukking van het gewas met een totale schade tot wel 7 miljard USD per jaar. De oogstverliezen veroorzaakt door dit onkruid brengen de voedselvoorziening van 300 miljoen Afrikanen in gevaar.

De immense schade veroorzaakt door *Striga* wordt mede veroorzaakt door het feit dat de beschikbare controlemaatregelen vaak ineffectief zijn. Mechanische maatregelen, zoals het handmatig verwijderen van de planten, kunnen vaak pas worden toegepast nadat de scheuten bovengronds zichtbaar zijn (na opkomst). Echter veel schade aan het gewas wordt al toegebracht in de eerste, ondergrondse, stadia van parasitisme, lang voordat de scheuten verwijderd zouden kunnen worden. Het is daarom noodzakelijk om nieuwe methodes te ontwikkelen die gericht zijn op deze vroege stadia van de parasiet, bij voorkeur zelfs nog voor de aanhechting.

Een cruciale stap in de levenscyclus van *Striga* is de kieming van de zaden. Deze kieming is strict gereguleerd, dat wil zeggen hij treedt alleen op in de aanwezigheid van strigolactonen, signaalstoffen die worden uitgescheiden door de wortel van de waardplant. Wij veronderstellen dat de strigolactonen een belangrijk handvat zouden kunnen zijn voor de controle van parasitaire planten. In mijn proefschrift bestudeer ik de relatie tussen strigolactonen en *Striga* infectie in graangewassen en onderzoek de mogelijkheden om deze kennis te gebruiken om de *Striga* schade te beperken. Hiertoe heb ik diverse aanpakken onderzocht (strigolacton biosynthese remmers, genetische variatie met betreking tot strigolacton productie en het effect van meststoffen op strigolacton productie) in laboratoriumstudies en – waar mogelijk – veldstudies in Kenia en Mali.

In eerder onderzoek hebben wij aangetoond dat de strigolactonen worden gemaakt vanuit carotenen. Mijn eerste hoofdstuk richt zich daarom op het gebruik van caroteen biosynthese remmers als middel om de strigolacton productie in de wortels van het gewas te remmen**.** In deze studie veronderstellen wij dat een (milde) remming van de caroteen biosynthese door deze biosynthese remmers, kan leiden tot gereduceerde strigolacton biosynthese (maar niet tot nadelige gevolgen voor het gewas) en daardoor verminderde *Striga* kieming en infectie. Extreem lage concentraties van vier verschillende caroteen biosynthese remmers (fluridone, norflurazon, clomazone and amitrole) werden door middel van irrigatie of bespuiting aan rijst toegediend. De strigolacton biosynthese werd inderdaad significant verminderd door irrigatie met alle caroteen biosynthese remmers, terwijl

bespuiting alleen effectief was voor amitrol. Een significante negatieve correlatie tussen de concentratie van de caroteen biosynthese remmer en *Striga* kieming én aanhechting werd gevonden voor fluridon, clomazon en norflurazon irrigatie. Toediening van caroteen biosynthese remmers zorgde voor een 61-75% reductie in *Striga* kieming, en een reductie met 65-90% van *Striga* aanhechting. Deze studie toont aan dat het remmende effect van caroteen biosynthese remmers (welke in vele male hogere concentraties als herbicide gebruikt worden) op strigolacton biosynthese en als gevolg daarvan op *Striga* kieming en aanhechting mogelijk ontwikkeld kan worden tot een goedkope, effectieve technologie om parasitisme door *Striga* tegen te gaan.

In het onderzoek in hoofdstuk 3 zochten we naar op lage strigolacton productie gebaseerde resistentie tegen *Striga*. Onze hypothese was dat gewas variëteiten met lagere strigolacton productie (partieel) resistent zijn tegen *Striga* doordat ze minder *Striga* kieming induceren. Om dit te onderzoeken werd een set van 18 hoogland rijst NERICA (New Rice for Africa) lijnen, en bijbehorende ouderlijnen, gescreend op strigolacton productie en andere *Striga* gerelateerde infectie parameters zoals; kieming, aanhechting, opkomst en *Striga* drooggewicht. NERICA 1 en CG14 produceerden significant minder strigolactonen en hadden minder *Striga* infecties, terwijl NERICAs 7, 8, 11 en 14 de hoogste strigolacton productie hadden en vele malen erger door *Striga* geïnfecteerd werden. Er bestond een significante positieve correlatie tussen de hoeveelheid strigolactonen en *Striga* infectie over de verschillende rijstlijnen heen. Deze studie toont aan dat de genetische variatie voor resistentie tegen *Striga* – gebaseerd op lagere kieming - in NERICA rijst het gevolg is van variatie in strigolacton productie. Dit feit kan van groot belang zijn voor veredelingsprogramma's gericht op de ontwikkeling van *Striga* resistente rijst variëteiten.

Aangetoond is dat strigolactonen ook een invloed hebben op de vertakking van planten. Hoe meer strigolactonen ze maken hoe minder vertakking. In hoofdstuk 4 testen we de hypothese dat in rijst variatie in strigolacton productie verbonden is met het aantal zijscheuten per plant en dat dit wederom gekoppeld kan worden aan de *Striga* infectie. In deze studie werd de genetische variatie voor strigolacton biosynthese, aantal zijscheuten en *Striga* infectie getest in een groot aantal rijstrassen van over de hele wereld verzameld. Rassen als IAC 165, IAC 1246, Gangweondo en Kinko produceren grote hoeveelheden strigolactonen, hebben weinig zijscheuten en induceren veel Striga kieming waardoor ze ook veel Striga infectie vertonen. In tegenstelling hiertoe werden in rijstrassen zoals Super Basmati, TN 1, Anakila and Agee lage strigolacton niveaus gemeten. Wortelexudaten van deze rassen induceerden ook lagere *Striga kieming*, en deze rassen hadden daardoor een lagere *Striga* infectie. Als gevolg van de lage strigolacton productie werd de uitgroei van zijscheuten in deze rassen extra gestimuleerd. Statistische analyse bevestigde een sterke positieve correlatie tussen strigolacton productie en *Striga* infectie en een negatieve relatie met de uitgroei van zijscheuten. Deze resultaten bevestigen dat genetische variatie in strigolacton productie resulteert in variatie in de uitgroei van zijscheuten en ook *Striga* infectie. Hierdoor kan mogelijk het excessief uitgroeien van zijscheuten

eenvoudig als indicatie worden gebruikt voor lage strigolacton productie in veredelingsprogramma's gericht op *Striga* resistentie.

Een aantal experimenten (hoofdstukken 5, 6, 7) zijn ontworpen met als doel de relatie tussen bemesting, strigolactonen en *Striga* kieming en infectie te kwantificeren. Mede hierdoor kan gezocht worden naar de mechanismen achter de geobserveerde reductie in *Striga* parasitisme als arme gronden worden bemest. Onze hypothese is dat de gereduceerde *Striga* infectie het gevolg is van lagere strigolacton uitscheiding naar de bodem door betere beschikbaarheid van minerale nutriënten. Onder kascondities werden verschillende stikstof (N) en fosfaat (P) niveaus toegediend aan rijst, maïs en sorghum en werden strigolacton productie en Striga infectie gekwantificeerd. Voor zowel maïs als sorghum werd ook een veldstudie uitgevoerd in Kenya en Mali in een poging om de in het kas/laboratorium verkregen resultaten te vertalen naar het veld. Niet alleen onderdrukt het toedienen van N en P *Striga* infectie in de kas bij alle drie de geteste gewassen effectief, ook correleerde de reductie in *Striga* infectie sterk met de reductie van in de bodem uitgescheiden strigolactone en *Striga* kieming geïnduceerd door de exudaten van deze gewassen.

De strigolacton productie verschilde ook sterk per ras. Zo produceert bijvoorbeeld het rijstras IAC 165 ongeveer 100x meer 2'-epi-5-deoxystrigol, orobanchol en drie nieuwe strigolactonen dan ras TN 1. Hoewel de resultaten van de veldexperimenten met maïs in Kenya minder consistent waren dan in de kasexperimenten, zeker in relatie tot het P effect, was er toch een duidelijke waarneembare trend waarbij bemesting *Striga* infectie reduceerde. De zogenaamde "microdosering" met de meststof diammonium phosphaat van in het veld gegroeide sorghum in Mali liet veel duidelijker zien dat *Striga* infectie onderdrukt kan worden door het toedienen van de juiste meststoffen. In microdosering wordt een kleine hoeveelheid meststof vlak bij het zaad in de grond gebracht. Deze resultaten tonen aan dat het positieve effect van meststoffen tegen *Striga* in ieder geval deels verklaard kan door een reductie in strigolacton biosynthese. Deze reductie heeft als consequentie dat *Striga* kieming, en daardoor aanhechting verlaagd wordt. Echter, verder onderzoek naar hoe meststoffen het best tegen *Striga* ingezet kunnen worden in het veld is nodig.

Samengevat kan worden geconcludeerd dat er een sterke correlatie bestaat tussen strigolactonen en kieming, aanhechting en biomassa van *Striga*. Deze correlaties kwam ik op het spoor door het gebruik van remmers van de strigolacton biosynthese, genetische variatie in strigolacton productie en door het toedienen van meststoffen. Ik ben van mening dat deze methodes kunnen worden ingezet om *Striga* infecties tegen te gaan tijdens de eerste fases van de levenscyclus van deze parasiet. Hoewel praktische toediening van deze methodieken nog verdere studie vereist, denk ik dat dit onderzoek kan leiden tot nieuwe componenten voor een effectieve, geïntegreerde aanpak van het *Striga* probleem.

خلاصہ

افریقہ میں اناج کی پیداوار میں ایک طفیلی جڑی بوٹی "سٹرائیگاہرمونتھیکا" (سکروولیری ایسی فیملی) کی وجہ سے مسلسل کمی ہورہی ہے۔ سٹرائیگاچری، باجرہ، مکئی اور چاول جیسے اناج کی جڑوں پر حملہ کرتی ہے۔ اس نے افریقہ کی تقریباً ۴۰ فیصد زرعی زمین کو نقصان پہنچایا ہے۔ یہ فصل کی مکمل ناکامی یا پیداوار میں شدید کمی کا باعث بنتی ہے جبکہ نقصان کی مالیت تقریباً ۷ ارب امریکی ڈالر فی سال ہے۔ اس جڑی بوٹی کی وجہ سے غریب کسان یا تقریباً ۳۰ کروڑ افریقی لوگ اپنی ۲۰ سے ۸۰ فیصد فصل کھو دیتے ہیں۔ سٹرائیگا کے اس قابل غور نقصان کی وجہ یہ حقیقت ہے کہ اس کی روک تھام کے موجودہ اقدامات غیر مؤثر ہیں۔ ان میں معاشرتی اور مشینی اقدامات، جیسے ہاتھ سے اکھاڑ پھینکنا شامل ہیں جو کہ اس جڑی بوٹی کے باہر آنے کے بعد اپنائے جاتے ہیں۔ چونکہ زیادہ تر نقصان طفیلی پن کے ابتدائی مراحل کے دوران زیر زمین ہوتا ہے لہٰذا ان کو جڑ سے چمٹنے یا زمین سے باہر آنے سے پہلے ہی تلف کرنے کی حکمت عملی تیار کرنے کی ضرورت ہے۔ اس جڑی بوٹی کو جڑ سے چمٹنے سے پہلے مرحلے میں تلف کرنے میں سٹرائیگو لیکٹون ایک اہم ایک اہم ہدف ہو سکتا ہے۔ سٹرائیگو لیکٹون کی بنیاد پر اگاؤ اور چمٹنے کے مراحل میں تلف کرنے کے طریقے زیادہ مؤثر ثابت ہو سکتے ہیں اور اناج کی فصل پر اس جڑی بوٹی کا حملہ کم کیا جا سکتا ہے۔ میں نے اپنے اس مقالہ میں اناج میں سٹرائیگو لیکٹون اور سٹرائیگا کے حملے میں تعلق کا مطالعہ کیا ہے اور سٹرائیگا کے اگاؤ اور چمٹنے کے مرحلے کے وقت نقصان کو کم کرنے کے مواقع دریافت کیے ہیں۔ اس کے لئے سٹرائیگا لیکٹون کے کم پیدا ہونے کے مختلف پہلوؤں جیسے مزاحمتی ادویات، جینیاتی تبدیلی اور کھادوں کے اثر کی لیبارٹری اور کینیا اور مالی کے کھیت میں تحقیقات کی تھیں۔

پہلی تحقیق میں پودوں کی جڑوں میں سٹرائیگو لیکٹون کی کمی کو بذریعہ مزاحمتی ادویات کے امکانات کو دیکھنا تھا۔ اس تحقیق میں ہم نے فرض کر لیا کہ کیرونائیڈ کی پیداوار میں ہلکی سی رکاوٹ سے سٹرائیگو لیکٹون اور سٹرائیگا کے اگاؤ اور حملہ میں کمی ہو سکتی ہے۔ چار مختلف قسم کی مزاحمتی ادویات (فلوریڈان، نارفلورازون، کلومازون اور امیٹرول) کی ہلکی سی مقدار چاول میں آب پاشی یا سپرے کے ذریعے ڈالی گئی تھی۔ آب پاشی کے ذریعے ڈالی گئی تمام مزاحمتی ادویات اور سپرے سے ڈالی گئی امیٹرول نے نمایاں طور پر سٹرائیگو لیکٹون کی پیداوار میں کمی کی تھی۔ سٹرائیگا کے اگاؤ اور چمٹنے پر فلوریڈان، نارفلورازون اور امیٹرول کی بذریعہ آب پاشی ایک نمایاں منفی تعلق دیکھا گیا جبکہ سپرے کے ذریعے ڈالی گئی امیٹرول نے سٹرائیگا کے اگاؤ میں نمایاں تعلق دکھایا ہے۔ مزاحمتی ادویات کے ڈالنے سے سٹرائیگا کے اگاؤ میں ۷۵-۶۱ فی صد اور چمٹنے میں ۹۴-۶۵ فی صد کمی ہوئی تھی۔ یہ تحقیق دکھاتی ہے کہ مزاحمتی ادویات کی کم مقدار (جو کہ زیادہ مقدار میں جڑی بوٹی مار ادویات کے طور پر استعمال ہوتی تھی) کا سٹرائیگو لیکٹون اور سٹرائیگا کے اگاؤ اور چمٹاؤ کے اثر کو ایک پر کشش سٹرائیگا کے روک تھام کی تکنیک کے طور پر تیار کیا جا سکتا ہے۔

دوسرے تجربہ (باب سوم) کا مقصد سٹرائیگا کے چمٹاؤ سے پہلے کی سٹرائیگو لیکٹون کی بنیاد پر مزاحمت کا اندازہ لگانا تھا۔ ہم نے فرض کر لیا کم سٹرائیگو لیکٹون پیدا کرنے والی فصلوں کی اقسام، بوجہ کم اگاؤ، سٹرائیگا کے چمٹاؤ سے پہلے کی مزاحمت کی مالک ہو سکتی ہے۔ اس مقصد کو پورا کرنے کے لئے نیریکا اور ان کے آبائی بیجوں کی اٹھارہ اقسام کی سٹرائیگو لیکٹون کی پیداوار اور سٹرائیگا کے حملہ کی بنیاد پر جانچ کی گئی۔ نیریکا-۱۱ اور سی جی-۱۴ نے سٹرائیگو لیکٹون اور سٹرائیگا کے حملہ میں نمایاں کمی دکھائی ہے۔ جبکہ نیریکا-۷، ۸، ۱۱ اور ۱۴ نے سب سے زیادہ سٹرائیگو لیکٹون کی پیداوار اور سٹرائیگا کے شدید حملہ کو ظاہر کیا ہے۔ چاول کی اقسام میں سٹرائیگو لیکٹون کی مقدار اور سٹرائیگا کے حملہ

کے درمیان مثبت تعلقات دیکھا گیا۔ یہ تجربہ نیریکا چاول میں سٹرائیگو لیکٹون میں جینیاتی تبدیلی کی وجہ سے سٹرائیگا کی چمٹنے سے پہلے کی مزاحمت کو دکھاتا ہے۔ یہ سٹرائیگا کے خلاف مزاحمتی اقسام کی ترقی کے پروگراموں کے لئے کافی زیادہ متعلقہ ہو سکتا ہے۔

باب چہارم میں ہم نے فرض کر لیا کہ سٹرائیگو لیکٹون کی پیداواری تبدیلی میں چاول کی شاخوں کے ساتھ باہم تعلق ہو سکتا ہے اور یہ تعلق سٹرائیگا کے حملہ کو متاثر کر سکتا ہے۔ اس تحقیق میں سٹرائیگو لیکٹون کی پیداوار اور سٹرائیگا کے حملہ اور چاول کی شاخوں کے جینیاتی تغیریات کو دیکھا گیا اور دنیا بھر سے چاول کی اقسام کا معائنہ کیا گیا۔ چاول کی اقسام جیسے آئی اے سی-۱۶۵، آئی اے سی-۱۲۴۶، گینگ ویوندو اور کنکو نے سٹرائیگا کا بہت زیادہ اگاؤ، چمٹاؤ، ابھار اور خشک مادہ دکھایا۔ اس کے برعکس چاول کی اقسام جیسے سپر باسمتی، ٹی این-۱، انا کیلا اور آگی نے سٹرائیگو لیکٹون کی کم پیداوار، کم سٹرائیگا اور زیادہ شاخیں دکھائی ہیں۔ چاولوں کی تمام اقسام کے سٹرائیگو لیکٹون کی پیداوار، سٹرائیگا کے حملہ اور شاخوں کے درمیان، شماریاتی تجزیہ ایک مضبوط مثبت تعلق کی تصدیق کرتا ہے۔ شاخوں کا ظاہری پن سٹرائیگو لیکٹون کی پیداوار میں اور سٹرائیگا کی مزاحمت کے عملی پروگرام میں ایک آسان اشارے کے طور پر استعمال کیا جا سکتا ہے۔

سٹرائیگو لیکٹون اور سٹرائیگا کے اگاؤ اور چمٹاؤ کے درمیان تعلق اور سٹرائیگا کے حملے میں ذمہ نظام کو دریافت کرنے کے لئے بہت سے تجربات (باب پنجم، ششم اور ہفتم) کئے گئے۔ ہم نے فرض کر لیا کہ بہتر معدنی غذائیت کی فراہمی، سٹرائیگو لیکٹون کے اخراج میں کمی کر کے سٹرائیگا کے حملہ کو کم کر دیتی ہے۔ نائٹروجن اور فاسفورس کی مختلف مقدار کو چاول، مکئی اور چری میں گرین ہاؤس حالات میں ڈالا گیا۔ گرین ہاؤس کے نتائج کھیت میں دیکھنے کے لئے چری اور مکئی پر کینیا اور مالی کے کھیتوں میں ایک متوازی مطالعہ کیا گیا۔ گرین ہاؤس کے تحت ڈالی گئی نائٹروجن اور فاسفورس نے تینوں فصلوں میں سٹرائیگا کے حملہ کو کم کر دیا اور سٹرائیگا کے اگاؤ کی کمی نے سٹرائیگو لیکٹون کی کمی کے ساتھ بھرپور مسابقت دکھائی۔ سٹرائیگو لیکٹون کی پیداوار میں بھی فصلوں کی اقسام کے لحاظ سے اختلاف تھا۔ چاول کی قسم آئی اے سی-۱۶۵ نے ٹی این-۱ کے مقابلے میں سو (۱۰۰) گنا زیادہ سٹرائیگو لیکٹون پیدا کیا۔ اگرچہ کینیا میں مکئی کے کھیت میں نتائج خاص طور پر فاسفورس کے گرین ہاؤس کے نتائج سے کم ملتے ہیں۔ پھر بھی کھاد نے سٹرائیگا کو کم کرنے کا رجحان دکھایا ہے۔ ڈائی امونیم فاسفیٹ کھاد کی کم مقدار نے بھی مالی میں چری کے کھیت میں سٹرائیگا کی قابل ذکر کمی کی ہے۔ جو کہ گرین ہاؤس میں سٹرائیگو لیکٹون کی پیداوار اور سٹرائیگا کے حملہ کے نتائج سے مسابقت رکھتے ہیں۔ یہ نتائج ظاہر کرتے ہیں کہ کھادوں کا سٹرائیگا کے خلاف مثبت اثر، کم از کم جزوی طور پر ہے جو کہ سٹرائیگو لیکٹون کی پیداوار میں کمی اور نتیجتاً سٹرائیگا کے اگاؤ اور چمٹاؤ میں کمی کی وجہ سے ہے۔ تاہم سٹرائیگا کے لئے کھیت میں کھاد کی مناسب مقدار کے تعین کے لئے مزید تحقیق کی ضرورت ہے۔

مجموعی طور پر یہ نتیجہ اخذ کیا جا سکتا ہے کہ سٹرائیگو لیکٹون اور سٹرائیگا کے اگاؤ، چمٹاؤ اور خشک مادہ کے درمیان ایک اچھا تعلق موجود ہے۔ ہم نے یہ مزاحمتی ادویات، جینیاتی تغیریات اور کھاد کا استعمال کر کے پایا ہے۔ ان ٹیکنالوجیز کو سٹرائیگا کے ابتدائی مرحلہ میں ہدف بنانے کے لئے ایک اہم آلہ کے طور پر استعمال کیا جا سکتا ہے اس حکمت عملی کو کھیت میں مزید عملاً تحقیق کی ضرورت ہے جو کہ سٹرائیگا کی روک تھام میں رہنمائی کر سکتی ہے اور سٹرائیگا کی مربوط روک تھام کا ایک موثر حصہ بن سکتی ہے۔

Abbreviations

%	:	Percent
µ	:	Micro
µg	:	Micro gram
um	:	Micro meter
uM	:	Micro molar
µM m^{-2} s^{-1}	:	Micro molar per meter square per second
ABA	:	Abscisic acid
a. i.	:	Active ingredient
ANOVA	:	Analysis of variance
AM	:	Arbuscular Mycorrhizal
^{o}C	:	Degree centi grade
CBSG	:	Centre for BioSystems Genomics
CCD	:	Carotenoid Cleavage Dioxygenase
CE	:	Collision energy
CID	:	Collision-induced dissociation
cm	:	Centimeter (s)
cv	:	Cultivar
d	:	Day
dSm^{-1}	:	Desi Semens per meter
DAS	:	Days after sowing
DMADP	:	dimethylallyl diphosphate
DM	:	Dry matter
DNMR	:	Duncan's New Multiple range test
ESI	:	Electro spray ionization
FAO	:	Food and Agriculture Organization
g	:	Gram (s)
GGDPS	:	Geranylgeranyl diphosphate synthase
GLM	:	Generalized linear model
g m-2	:	Grams per meter square
h	:	Hour
ha^{-1}	:	Per hectare
IDP	:	Isopentenyl diphosphate
IPPI	:	Isopentenyl diphosphate isomerase
K	:	Potassium
Kg	:	Kilogram (s)
L	:	Liter
LC	:	Liquid Chromatography
M	:	Molar
MCP	:	Monte Carlo permutation
mm	:	Millimeter
mL	:	Milliliter
m^{-2}	:	Per meter square
min	:	minute
MS	:	Mass Spectrometry
MRM	:	Multiple Reaction Monitoring
N	:	Nitrogen
NCED	:	9-*cis*-epoxycarotenoid dioxygenase
NERICA	:	New Rice for Africa
NH_4	:	Ammonium
NO_2	:	Nitrite

NO_3	:	Nitrate
NS	:	Non significant
P	:	Phosphorus
P	:	Probability level
PA	:	Peak area
PC	:	Principal Component
PDS	:	Phytoene desaturase
$Plant^{-1}$	:	Per plant
ppm	:	Parts per million
PS	:	Phytoene synthase
RCD	:	Randomized complete design
RDA	:	Redundancy analysis
SD	:	Standard deviation
$t\ ha^{-1}$	:	Tonne (s) per hectare
UPLC	:	Ultra performance liquid chromatography
US$	:	United State Dollar
WAP	:	Week after planting
ZD	:	ζ-carotene desaturase

Statement concerning data

The replicated raw data, details of analysis have been lodged in the Laboratory of Plant Physiology, Wageningen University and Research Center, the Netherlands. Any person interested may approach to Prof. & Chair. dr. Harro J. Bouwmeester (harro.bouwmeester@wur.nl) for the use of data.

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For better efficiency in lab work it is important that you have nice company and a friendly environment to spend good and refreshing time outside of it. I was blessed with plenty of nice friends in Wageningen and other cities of the Netherlands. First of all I like to admire sincere initial help by Muhammad Ali and Faisal Bhai in Nijmegen. These nice persons welcomed and received us on the airport, took us in Nijmegen and helped us in our adjustment in Netherlands. I like to mention the nice moments spent with Mazhar Iqbal Zafar, Syed Hamid Ali Shah, Masood Awan, Kashif, Abid, and Akmal; family gathering with Dr. Sajid Rehman, Hafiz Sultan Mahmood, Munawar Shah, Abdul Mateen, and some other Pakistani community in Wageningen. In addition to these, I would also like to thank all other colleagues and friends in different parts of Netherlands. I cannot include everybody's name as the list is very long. I also enjoyed living in Wageningen, the city of life sciences. It was great to learn about a diverse spectrum of cultures here.

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Now I would like to thank my parents, parents in law, brothers (Muhammad Shafique, Muhammad Aslam, Muhammad Arshad, and Muhammad Ali), sisters and sisters in law for their unlimited prayers and best wishes for my success. I like to mention my son Muhammad Ahsan Jamil, who strived a lot to recover from his medical complications. Thanks God now he is enjoying a good, normal life. I also like to say my lovely comments to my daughter Omamah Khalud Jamil. She tried to survive in Wageningen without our much assistance. I would like to apologies for being late so many times to pick her up from school and very limited time spare for her to play.

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About the author

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2nd February, 1974
(Faisalabad, Pakistan)

Muhammad Jamil was born on 2nd February 1974 in a village of Faisalabad, Pakistan. Since 1997, he attained a Bachelor of Science degree in Department of Agronomy, University of Agricultural Faisalabad, Pakistan. Within next two years in 1999, he completed his Master of Science degree in Agronomy from same Department. After that he was selected as Scientific Officer in a Research Organization "Pakistan Agricultural Research Council" where he gained experience in genotypic evaluation and development of Sugar Crops in Pakistan. After five years, Higher Education Commission of Pakistan selected him for Ph. D. study in Netherlands. Prof. Harro J. Bouwmeester, chair Lab. of Plant Physiology, accepted him as Ph. D. candidate to work in a vici Project on parasitic plants, financed by Netherlands Organization for Scientific Research (NWO).

His significant achievements include a number of scientific publications with more than 60 impact factors, presentation in International Conferences, *Striga* field visit in Africa, Articles in National News Paper, Technical Reports and Production Technology on Sugar and Cereal Crops, Certificate of Outstanding Performance in education from Government of Pakistan, Silver Medal in Secondary School Certificate examination, Quide-e-Azam scholarship in F. Sc., President Rover Scouting. His wife being a Ph. D. in Statistics has contributed to make a genius family setup at home and last not least he is proud father of two smart kids. His future planning is to re-join his parent department in Pakistan and after that to do some post doc to enhance his further research experience.

List of Publications

Alder A, **Jamil M**, Marzorati M, Vermathen M, Bigler P, Ghisla S, Bouwmeester H, Al-Babili S (2011) Two oxygenases convert 9-cis-β-carotene into carlactone, a new strigolactone like plant hormone. Science (under review)

Jamil M, Rodenburg J, Charnikhova T, Bouwmeester H (2011) Pre-attachment *Striga hermonthica* resistance of NERICA cultivars based on low strigolactone production. New Phytologist 192: 964-975.

Jamil M, Charnikhova T, Houshyani B, Ast A, Bouwmeester H (2011) Characterization of relationship between strigolactones and tillering phenotype of some global rice cultivars influencing *Striga hermonthica* infection. Planta: DOI 10.1007/s00425-00011-01520-y

Jamil M, Charnikhova T, Cardoso C, Jamil T, Ueno K, Verstappen F, Asami T, Bouwmeester HJ (2011) Quantification of the relationship between strigolactones and *Striga hermonthica* infection in rice under varying levels of nitrogen and phosphorus. Weed Research 51: 373-385

Jamil M, Karaya H, Charnikhova T, Fred K, Bouwmeester H (2011) *Striga hermonthica* parasitism in maize in response to N and P fertilizers. Field Crop Research (Accepted)

Jamil M, Van Mourik TA, Charnikhova T, Bouwmeester HJ (2011) Fertilizer microdosing for *Striga hermonthica* control in sorghum. Weed Research (under review)

Liu W, Kohlen W, Lillo A, Camp RO, Ivanov S, Hartog M, Limpens E, **Jamil M**, Smaczniak C, Kaufmann K, Yang WC, Hooiveld GJEJ, Charnikhova T, Bouwmeester HJ, Bisseling T, Geurtsm R (2011) Strigolactone biosynthesis requires the symbiotic gras-type transcription factors NSP1 and NSP2. Plant Cell doi: 10.1105/tpc.111.089771

Malik H, Kohlen W, **Jamil M**, Rutjes F, Zwanenburg B (2011) Aromatic A-ring analogues of orobanchol, new germination stimulants for seeds of parasitic weeds. Organic & Molecular Chemistry 9: 2286.

Jamil M, Charnikhova T, Van Mourik TA, Kanampiu F, Bouwmeester HJ (2011) Unraveling the mechanism involved in *Striga* parasitism in cereals under nutrient deficient conditions. 11th world Congress on Parasitic Plants 7-12 June 2011, Martina Franca, Italy pp. 64.

Cardoso C, **Jamil M**, Hepworth J, Zhang Y, Charnikhova T, Leyser O, Dimkpa SON, Reiff C, Price AH, Bouwmeester HJ, Ruyter-Spira C (2011) Deletion of a MAX1 ortholog involved in strigolactone biosynthesis, occurs as a natural variation among rice cultivars. PNAS (In Prep.)

Cardoso C, Charnikhova T, **Jamil M**, Verstappen F, Delaux PM, Lauressergues D, Ruyter-Spira C, Yoneyama K, Bouwmeester H (2011) Differential stimulation of *Striga hermonthica* germination and arbuscular mycorrhiza hyphal branching by rice root exudates. New Phytologist (In prep.)

Jamil M, Charnikhova T, Verstappen F, Bouwmeester H (2010) Carotenoid inhibitors reduce strigolactone production and *Striga hermonthica* infection in rice. Archives of Biochemistry and Biophysics 504: 123-131

Jamil M, Cheema ZA, Mushtaq MN, Farooq M, Cheema MA (2009) Alternative control of wild oat and canary grass in wheat fields by allelopathic plant water extracts. Agronomy for Sustainable Development 29: 475-482

Jamil M, Charnikhova T, Cardoso C, Kohlen W, Ruyter-Spira C, Verstappen F, Bouwmeester H (2009) Quantifying the relationship between strigolactones and *Striga hermonthica* under varying levels of nitrogen and phosphorus in rice (*Oryza sativa*). In: Rubiales D, Westwood J, Uludag A (eds) 10th World Congress on parasitic plants, Kusadasi, Turkey, p 68

Lopez-Raez JA, Matusova R, Cardoso C, **Jamil M**, Charnikhova T, Kohlen W, Ruyter-Spira C, Verstappena F, Bouwmeestera, H (2008) Strigolactones: ecological significance and use as a target for parasitic plant control. Pest Management Science 64: 471–477

Chattha MR, **Jamil M**, Mahmmod TZ (2007a) Yield and yield components of cowpeas as affected by various weed control methods under rainfed conditions of Pakistan. International Journal of Agriculture & Biology 9: 120-124

Chattha MR, **Jamil M**, Mahmmod TZ (2007b) Yield and yield components of mungbean as affected by various weed control methods under rainfed conditions of Pakistan. International Journal of Agriculture & Biology 9: 114-119

Jamil M, Majid MA (2007) Performance of sugarcane varieties in National Uniform Yield Trials. Pakistan Sugar Journal XXII 61-72

Riaz M, **Jamil M**, Mahmmod TZ (2007) Yield and yield components of maize as affected by various weed control methods under rainfed conditions of Pakistan. International Journal of Agriculture & Biology 9: 152-155

Riaz M, Malik MA, Mahmmod TZ, **Jamil M** (2006) Weed management in wheat based cropping patterns under rainfed conditions. International Journal of Agriculture & Biology 8: 636-640

Akhtar M, **Jamil M**, Ahmad S (2006) Agronomic traits and morphological characteristics of some exotic varieties of sugarcane. Pakistan Journal of Agricultural Research 19: 70-77

Jamil M, Ata Z, Khaliq A (2006) Development of suitable strategies for the economic control of *Avena fatua* and *Phalaris minor* in wheat. International Journal of Agriculture and Biology 7: 719-723

Jamil M, Ata Z, Khaliq A (2005) Increasing the efficiency of sorghum water extract (sorgaab) by mixing with lower doses of isoproturon to control weeds in wheat. International Journal of Agriculture and Biology 7: 712-718

Jamil M, Gopang AD (2004) Sugarcane scenario, constraints and strategies to improve its production in Pakistan. Pakistan Sugar Journal 109: 55-67

Khan I, **Jamil M** (2004a) Prospectus of sugar beet cultivation in the Punjab and Sindh. Science, Technology and Development 23: 51-55

Khan I**, Jamil M** (2004b) Sugar crops and sugar policy of Pakistan. Science Vision 9: 1-8

Khan I, **Jamil M**, Tanveer, Jalal Y (2001) Sugar beet cultivation/promotion in Punjab and Sindh to supplement sugarcane. 36th Annual Convention of the Pakistan Society of Sugar technologist, Lahore, Pakistan., pp 11-26

Jamil M, Hussain A (2000) Effect of different planting methods and nitrogen levels on growth and yield of rice Basmati – 385. Journal of Agricultural Science 37: 83-85

Education Statement of the Graduate School

Experimental Plant Sciences

Issued to: Muhammad Jamil
Date: 11 January 2012
Group: Plant Physiology, Wageningen University and Research Centre

1) Start-up phase	*date*
► **First presentation of your project**	
Managing germination stimulants biosynthesis: an indirect approach to overcome Striga hermonthica	Nov 07, 2007
► **Writing or rewriting a project proposal**	
Managing germination stimulants biosynthesis: an indirect approach to overcome Striga hermonthica	Nov 2007
► **Writing a review or book chapter**	
► **MSc courses**	
PBR-20306 Plant Biotechnology	Oct 29-Dec 17, 2007
MOB-20306 Gene Technology	Apr 28-Jun 16, 2008
► **Laboratory use of isotopes**	
Subtotal Start-up Phase	*9,5 credits**

2) Scientific Exposure	*date*
► **EPS PhD student days**	
EPS PhD student day, Wageningen University	Sep 13, 2007
EPS PhD student day, Leiden University	Feb 26, 2009
EPS PhD student day, Utrect University	Jun 01, 2010
► **EPS theme symposia**	
EPS theme 3 symposium 'Metabolism and Adaptation', Amsterdam University	Feb 18, 2009
EPS theme 3 symposium 'Metabolism and Adaptation, Leiden University	Feb 19, 2010
► **NWO Lunteren days and other National Platforms**	
ALW meeting 'Experimental Plant Sciences', Lunteren	Apr 07-08, 2008
ALW meeting 'Experimental Plant Sciences', Lunteren	Apr 06-07, 2009
ALW meeting 'Experimental Plant Sciences', Lunteren	Apr 19-20, 2010
► **Seminars (series), workshops and symposia**	
European Flying Seminar "Regulation of phase change in plants by miRNAs and trans-acting siRNAs " by prof. dr. Scott Poethig	Sep 24, 2007
Symposium "Plant Roots: from Genes to Ecosystems" Radboud University, Nijmegen	Oct 23, 2008
EPS Symposium "Ecology and experimental plant science 2" WUR, Wageningen	Sep 22, 2009
Career Expectation day	Nov 19, 2010
► **Seminar plus**	
► **International symposia and congresses**	
10th World Congress on Parasitic Plants Kusadasi, Turkey	Jun 08-12, 2009
11th World Congress on Parasitic Plants Matina Franca, Italy	Jun 07-12, 2011
3rd Joint retreat of PhD Students in Plant Sciences, Orsay University Paris France	Jul 05-08, 2011
► **Presentations**	
Poster Presentation: EPS PhD student day, Leiden University	Feb 26, 2009
Poster Presentation: ALW meeting 'Experimental Plant Sciences', Lunteren	Apr 06-07, 2009
Poster Presentation: 10th World Congress on Parasitic Plants Kusadasi, Turkey	Jun 09, 2009
Oral presentation: ALW meeting 'Experimental Plant Sciences', Lunteren	Apr 07, 2009
Oral presentation: 10th World Congress on Parasitic Plants Kusadasi, Turkey	Jun 09, 2009
Oral Presentation, Theme 3 symposium, Leiden University	Feb 19, 2010
Poster Presentation: EPS PhD student day, Utrecht University	Jun 01, 2010
Oral presentation: 11th World Congress on Parasitic Plants Matina Franca, Italy	Jun 10, 2011
Poster Presentation: Ph. D. retreart, Orsay University, Paris France	Jul 07, 2011
► **IAB interview**	Dec 04, 2009
► **Excursions**	
Subtotal Scientific Exposure	*15,9 credits**

3) In-Depth Studies	*date*
► **EPS courses or other PhD courses**	
Summer School 'Rhizosphere Signaling'	Aug 23-25, 2010
Basic Statisics	Dec 15-17 & 21-22, 2009
► **Journal club**	
Member of a literature discussion group at Plant Physiology	2007-2011
► **Individual research training**	
Subtotal In-Depth Studies	*5,4 credits**

4) Personal development	*date*
► **Skill training courses**	
Ph. D. Competence Assesment	Mar 18, 2008
Techniques for Writing and Presenting a Scientific Paper	Dec 09-12, 2008
Interpersonal Communication for PhD Students	Oct 27-28, 2008
Scientific writing	Oct-Nov 2010
Career perspective	Mar-Apr, 2011
Writing grant proposals	Sep-Nov 2011
► **Organisation of PhD students day, course or conference**	
► **Membership of Board, Committee or PhD council**	
Subtotal Personal Development	*7,4 credits**

TOTAL NUMBER OF CREDIT POINTS*	**38.2**

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of EPS which comprises of a minimum total of 30 ECTS credits